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Influence of Farm Dairy Effluent Ammonium Concentrations on Soil N_2O Emissions

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A Dissertation

submitted in partial fulfilment

of the requirements for the Degree of

Bachelor of Agricultural Science (Honours)

at

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by

Tony Mark Johnston

Lincoln University

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science (Honours).

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by

Tony Mark Johnston

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) and the single-most ozone (O_3) depleting substance. Agriculture is the dominant source of anthropogenic N_2O emissions globally, and especially in New Zealand. Urine, synthetic nitrogen (N) fertiliser and farm dairy effluent (FDE) are the main sources of N_2O emissions from agricultural soils in New Zealand. Urine and synthetic N fertiliser have received considerable research attention to minimise their contribution to soil N_2O emissions due to the high N loadings and greater emission factors (EF) of these inputs. However, as the land application of Farm Dairy Effluent (FDE) is a less significant contributor to New Zealand's overall N_2O emissions profile, research on this N-input is limited. The mass of FDE applied to land increased from, 18kt in 1990 to 39kt in 2013, and a recent increase in popularity of herd homes will further increase the mass of FDE produced. Thus, FDE requires further research.

Limited data is available on the EF's from the land application of FDE. In additon, an analysis of the literature suggests the NH_4^+-N concentration of FDE is highly variable. The aim of this study was to determine the influence of FDE NH_4^+-N concentration on soil N_2O emissions. A 35-day field trial was conducted, where 10 mm of FDE was applied to pasture at NH_4^+-N concentrations of either 90, 150, 200, 300 or 400 mg NH_4^+-N L^{-1}. The 150 and 400 mg NH_4^+-N L^{-1} treatments contained ^{15}N to monitor the fate of FDE NH_4^+-N.

N_2O gas samples were taken daily for the first week, then every 2-3 days for the remainder of the trial. Soil inorganic-N pools were monitored every 7 days. Pasture production was measured on days 19 and 35.

Peak N_2O emissions occurred within 24 hrs of applying the FDE. The highest N_2O emisions were produced in the 400 mg NH_4^+-N L^{-1} treatment averaging 65 kg N_2O ha^{-1} day^{-1}. FDE treatments produced significantly more than the control until day 7. Emission factors ranged from 0.18 to 0.32

percent of total N applied, significantly less than the 1% currently used to calculate New Zealands GHG inventory. The emission period was relatively short due to low soil nitrate concentrations, and/or relatively dry soil conditions. Ammonium concentration is a key driver of N_2O emissions. Cumulative soil N_2O emissions increased linearly with FDE NH_4^+-N concentration, however, it is likely this relationship may change in soils with a higher moisture content. Therefore, the use of a single relationship between the two variables for all environmental situations may not be possible. Further studies that analyse the influence of NH_4^+-N concentrations on soil N_2O emissions, in a range of typical environments, are required to fully understand this relationship.

Keywords: Ammonium, NH_4^+, Farm dairy effluent, FDE, Effluent, Emissions, Emission factor, Nitrogen, Nitrous oxide, N_2O, Pasture, Perennial ryegrass, Rate, White clover.

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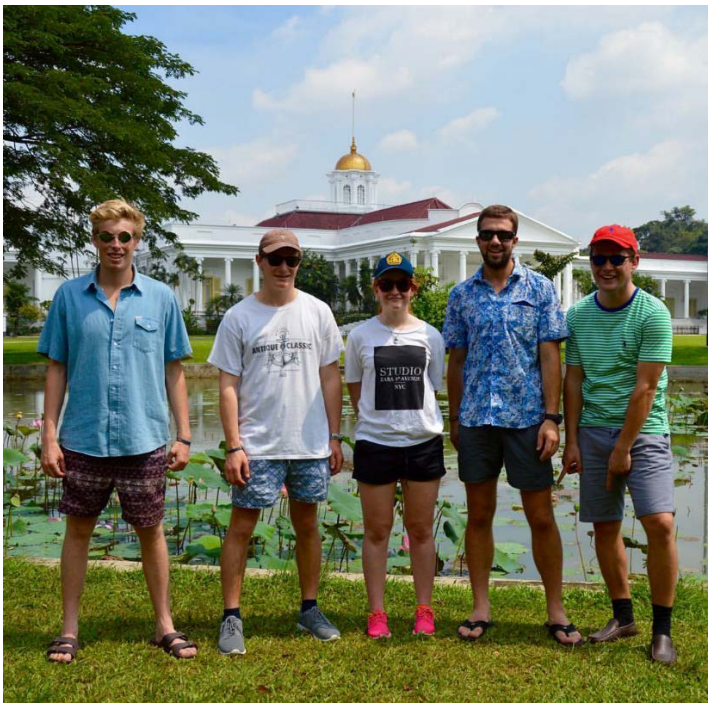

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

A review of the literature found that there are very few measurements of nitrous oxide (N_2O) fluxes from the application of farm dairy effluent (FDE) to typical perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) pasture in New Zealand. Currently FDE application is regulated by application depth and total nitrogen (N) loading, however, these regulations are aimed at minimising nitrate (NO_3^--N) leaching, with no consideration of gaseous emissions such as N_2O. Chemical characteristics of FDE are highly variable, particularly the ammonium (NH_4^+-N) concentration. It has been suggested that NH_4^+-N concentration of the FDE drives N_2O emissions, as against total N concentration. However, any potential relationship between the level of N_2O emissions and the concentration of NH_4^+-N in the FDE applied is not clear, as no research has been conducted to confirm this. Understanding the key factors driving N_2O emissions is an essential step towards both, mitigating N_2O emissions and accurately predicting them for calculation of New Zealand's greenhouse gas inventory. The objective of this research was to analyse how FDE NH_4^+-N concentration influenced soil N_2O emissions when applied to perennial ryegrass/white clover grazed pasture.

Chapter 2

Literature Review

2.1 Introduction

Nitrogen (N) and the N cycle are pivotal to agriculture in New Zealand. An essential element for plants, N is typically the most limiting nutrient to their growth. Plants obtain their N from the soil as either ammonium (NH_4^+-N) or nitrate (NO_3^--N) with a strong preference for NO_3^--N. However, the form of N is important, only 1-2% of the total N in the soil is available to plants as NH_4^+-N and NO_3^--N, up to 95% is bound as organic compounds in soil organic matter (SOM). The remaining 1-6% of N in soil is unavailable NH_4^+-N, fixed by clay minerals. Nitrogen can be added to the soil-plant system through the addition of fertilizers, biological N fixation, animal manure and atmospheric returns. However, of concern environmentally and economically are the losses of N from the soil through volatilization, NO_3^--N leaching and denitrification. A basic N cycle can be found in Figure 2-1.

An important loss of N is the evolution of nitrous oxide (N_2O). Nitrous oxide is a potent greenhouse gas contributing to global warming and destruction of the ozone (O_3) layer. Agriculture is a major contributor of N_2O, especially in New Zealand. Intensification of agriculture due to advances in technology and increasing pressure to feed the growing population has exacerbated agriculture's contribution to total N_2O emissions. Dairying is a significant contributor to New Zealand's agricultural N_2O emissions due to the large number of dairy farms and high concentration of N involved. Fertiliser, urine and farm dairy effluent (FDE) are the three main inputs of N on a dairy farm. Fertiliser and urine are applied to pastures at high rates and are considered the most important N inputs in terms of N_2O emissions and other N losses. This has led to a large amount of research on these inputs and associated losses of N. However, FDE has received much less attention as it is a less concentrated form of N and thought to produce less emissions. Despite being a less concentrated form of N, a large volume of FDE is produced from dairy sheds in New Zealand and applied to pastures each year. Therefore, FDE is worthy of further research to understand the processes and magnitude of N_2O emissions.

Currently regulations governing the application of FDE to pasture are based around application depth and total N concentrations to minimise NO_3^--N leaching. No consideration is given to the influence of FDE on N_2O emissions. Chemical characteristics of FDE are highly variable, especially the NH_4^+-N concentration. Chadwick *et al.* (2011) indicated it may be the available N (NH_4^+-N and NO_3^--N) concentration of FDE, not the total N concentration that drives N_2O emissions. This is in agreement

with the current general understanding that NH_4^+-N and NO_3^--N are critical substrates for N_2O production. There is also a lack of information on the effect of increasing N applications on N_2O emissions. Therefore, this research will analyse the effects of solely varying the available N content of FDE, specifically the NH_4^+-N content, on N_2O emissions. Nitrate concentration of FDE is normally negligible. The research will help to determine if FDE NH_4^+-N concentration is a key driver of N_2O emissions. This will increase our understanding of N_2O emissions, which is a critical step towards developing strategies for their mitigation. An improved understanding, may ultimately result in regulations governing the land application of FDE in New Zealand being updated, to encompass strategies to minimise N_2O emissions. The research will also provide information on the N_2O EF's from FDE, to aid in the calculation of New Zealand's greenhouse gas inventory. It is expected that N_2O emissions will increase linearly with NH_4^+-N content.

2.2 N Cycle

There are several gains and losses from the N cycle as can be observed in Figure 2-1.

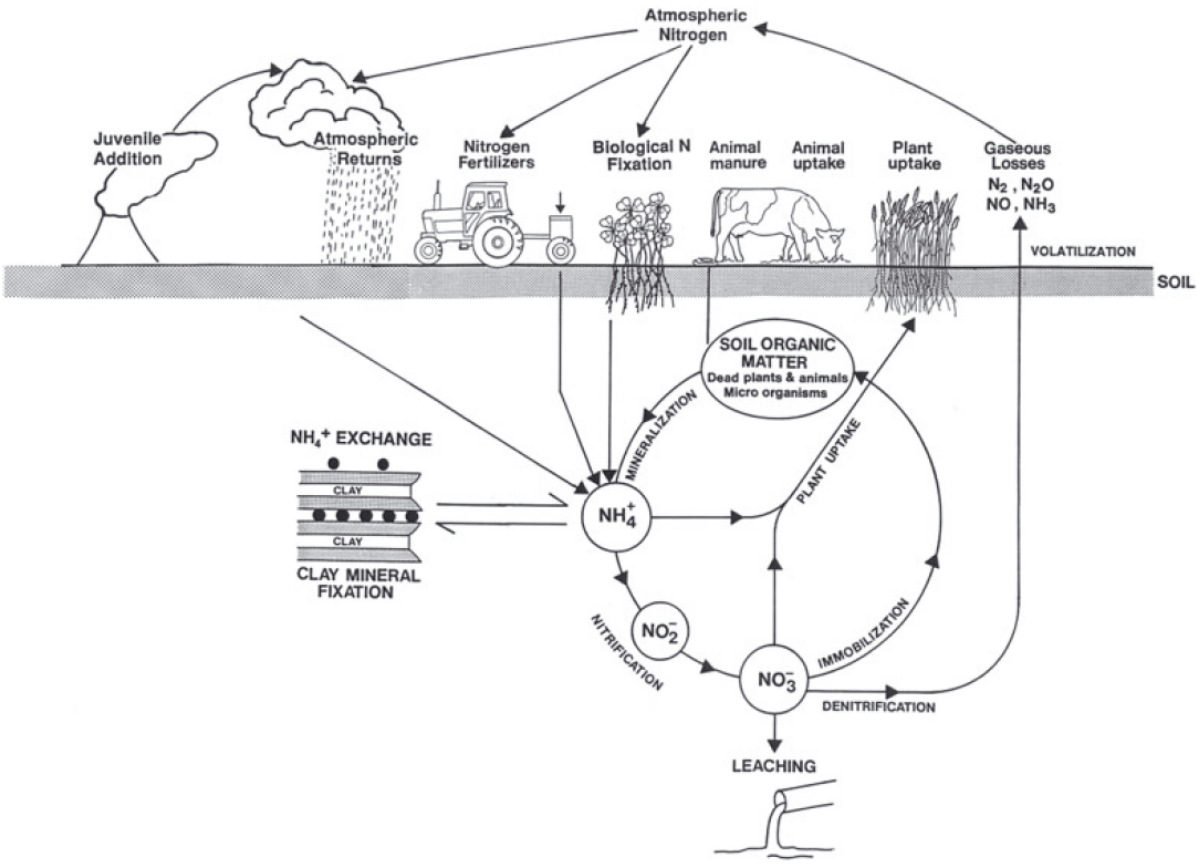

Figure 2-1 The soil/plant nitrogen cycle, from Cameron *et al.* (2013).

2.2.1 Gains

Nitrogen inputs to the soil plant system originate from molecular dinitrogen (N_2) in the atmosphere (Figure 2-1). Nitrogen must be fixed to enter the soil-plant system, only biological N fixation, lightening and anthropogenic fertilizer production have sufficient energy for this to occur (Haynes *et al.*, 1986). All other N inputs are losses that have been recycled.

Fertilizer

Industrial fixation of N, enabling the production of nitrogenous fertilizers, has increased to 92 Tg yr^{-1} (FAO, 2015). Such anthropogenic inputs have dramatically increased crop yields since the mid 1900's. Intensive agricultural systems are now reliant on large inputs of fertilizer N to sustain high levels of production (Haynes *et al.*, 1986). In New Zealand, the average dairy farm applies 120 kg N ha^{-1} $year^{-1}$ of commercially synthesised ammonia (NH_3), an increase from almost zero in the 1960's (MPI, 2012).

Small quantities of natural sodium nitrate are used as fertilizer N, however, over 80% is from the commercial synthesis of NH_3.

Ammonia is produced by the Haber Bosch process, through the equation:

$$N_2 + 3H_2 \rightarrow 2NH_3 \quad \textbf{(1)}$$

Nitrogen and Hydrogen are combined at elevated temperature (300 and 500°C) and pressure (400 to 1000 atmospheres) in the presence of a catalyst such as reduced iron (Haynes *et al.*, 1986).

Biological Fixation

Biological N fixation is the conversion of atmospheric N_2 by micro-organisms to organic N compounds. There are two systems of biological N fixation: (i) fixation by free living microorganisms and (ii) fixation by micro-organisms, which live in symbiosis with higher plants. Both systems are similar; micro-organisms reduce N_2 to NH_3 in a reaction involving the nitrogenase enzyme:

$$N_2 + 6e^- + 6H^+ \xrightarrow{nitrogenase\ enzyme} 2NH_3 \quad \textbf{(2)}$$

Symbiotic N fixation is major biological source of N in agricultural systems. Bacteria of the genus *Rhizobium* invade the roots of legumes and develop a nodule where they live and fix N_2. The host plant provides the bacteria with carbohydrates for energy and the plant receives NH_3 in return. Several strains of Rhizobium are available and each has a preferred host plant. E.g. Rhizobium *trifolli* fixes N most efficiently when in symbiosis with clovers (*Trifollium*) (Haynes *et al.*, 1986; McLaren and Cameron, 1996).

The amount of N fixed by micro-organisms depends on a large number of factors including moisture, oxygen (O_2) concentration, temperature, pH and carbon (C) and nutrient availability (Haynes *et al.*, 1986). On average 184 kg N ha^{-1} $year^{-1}$ is fixed by clovers, with considerable variation (107 – 392 kg N

ha^{-1} $year^{-1}$) depending on soil fertility, moisture status, grazing management, temperature and the pasture's clover content (Hoglund *et al.*, 1979). Estimated rates of N_2 fixed by various legumes and free living organisms can be found in Table 2-1.

Table 2-1 Estimated amounts of N_2 fixed by legumes and free living organisms, from McLaren and Cameron (1996).

	Amount of N_2 fixed (kg ha^{-1} $year^{-1}$)
Legumes	
Clover	100-200
Lucerne	125-600
Lupins	150
Free-living micro-organisms	
Blue-green algae	25
Azotobacter	0.3
Clostridium	0.5

Mineralization of fixed organic N in "sloughed off" roots and nodules of legumes can provide a source of N to plants e.g. perennial ryegrass grown in association with the legumes. Similarly, the organic matter of legumes will be mineralized, releasing N when the plants are killed by ploughing or through herbicide application. In a mixed pasture, N fixed by clovers will be made available to the grass component in the form of animal excreta (Haynes *et al.*, 1986; McLaren and Cameron, 1996). In contrast, some free-living micro-organisms e.g. Blue-green algae can fix N_2 without relying on higher plants. Some obtain their energy from reactions with C or carbon dioxide (CO_2), while others rely on light energy. Azotobacter and clostridium are free living bacteria capable of fixing N_2 in soil. The amount of N fixed is variable and is only made available once the organism dies and is decomposed (Haynes *et al.*, 1986).

Animal Manure

Animal manure can be returned to the soil directly, when animals graze pasture, or it can be collected when animals are milked in a dairy shed or housed in a barn and applied to pastures later. Nitrogen concentrations in forage are well in excess of animal requirements leading to a high return of N via excreta (Haynes *et al.*, 1986). Return of N from animals grazing a pasture is non-uniform, excreta is often deposited in gateways, stock camps and on stock tracks. In addition, a urine patch from a sheep may contain up to 500 kg N ha^{-1}, but this may double to 1000 kg N ha^{-1} for a dairy cow's urine patch (Di and Cameron, 2002b). Most N in urine is urea [$CO(NH_2)_2$], which rapidly hydrolyses to NH_4^+-N in the soil. In contrast, the majority of N in dung is organic N, which is slowly made available by mineralisation (Haynes *et al.*, 1986).

Waste that is collected, stored and applied later is highly variable in chemical characteristics as highlighted in

Table 2-7. An animal's diet, quantity of wash-down water used and the length of time manure is stored, all influence such characteristics (Longhurst *et al.*, 2000).

Atmospheric Returns

Dinitrogen (N_2) comprises 78% of the atmosphere. In addition to N_2, trace amounts of N oxides (NOx), nitric acid (HNO_3) vapour, particulate NO_3^--N, gaseous NH_3, NH_4^+-N compounds and organic N are present in the atmosphere. Such compounds arrive in the atmosphere naturally as emissions from soil, vegetation and wildfires, however, contributions of N from anthropogenic sources such as agriculture, industry and transportation now exceed those from natural sources (Haynes *et al.*, 1986; Asman *et al.*, 1998a; Vitousek *et al.*, 1997).

Atmospheric returns are inputs of N compounds from the atmosphere to the biosphere. Such compounds can enter the biosphere via wet or dry deposition. Wet deposition occurs when rain or snow carry gases or particles down to the soil surface. The concentration of N deposited by wet deposition depends not only on the amount of N in the atmosphere, but also on the amount of precipitation. Dry deposition is more complex, it occurs when particles settle to a surface, collide with, and attach to a surface or when gases bind to a surface (adsorption) or are absorbed. Wet deposition can occur a long distance from the source, whilst dry deposition occurs in close vicinity to the source.

Nitric oxide (NO) and N dioxide (NO_2) (combined term NOx) are emitted to the atmosphere from emissions of fossil fuel combustion. The heat involved has sufficient energy to oxidise N_2. Nitric oxide is then quickly oxidised by atmospheric O_3 to NO_2. Nitrogen oxides have a short atmospheric life; they normally only remain in the atmosphere for 1-2 weeks. A large amount of NO_2 is hydrolysed to HNO_3, of which the HNO_3 vapour can condense and be incorporated into existing aerosols in the atmosphere and is deposited mostly by wet deposition. Nitric oxide and NO_2 can also be removed from the atmosphere by dry deposition, sorbed by soil or removed by vegetation (Haynes *et al.*, 1986).

In contrast, agriculture is the main source of reduced N. Ammonia gas is volatilised when fertiliser, urine or animal manure is added to the soil (Asman *et al.*, 1998b).

Ammonia readily dissolves and ionises to NH_4^+-N in atmospheric water vapour, which reacts with acid pollutants in the atmosphere, producing NH_4^+-N aerosols containing NH_4^+-N salts (Asman *et al.*, 1998a). Normally aerosols are returned to the Earth's surface by wet deposition, however, if evaporation occurs then N particles in the aerosol may be returned by dry deposition. Unreacted NH_3 is also returned by dry deposition (Haynes *et al.*, 1986).

Nitrous oxide produced from denitrification in the soil has an extremely long stratospheric lifetime of 100 – 150 years. No significant removal of atmospheric N_2O from the troposphere occurs from precipitation due to its low solubility in water. Therefore, N_2O can penetrate almost unimpeded into

the stratosphere. Photochemical reactions in the stratosphere are the only means of atmospheric destruction of N_2O (Haynes *et al.*, 1986). Ninety percent of N_2O in the stratosphere is converted to N_2, the remainder is transformed to NO through the reaction:

$$N_2O + O('D) \rightarrow 2NO \quad \textbf{(3)}$$

O('D) is an electronically excited atom produced by photolysis of O_3 in the stratosphere (Haynes *et al.*, 1986).

Further atmospheric effects of N_2O, including its role in stratospheric O_3 depletion, will be discussed in section 2.3.1.

The NO produced in the destruction of N_2O is quickly oxidised to NO_2 and hence to HNO_3. These substances are then eventually returned to the biosphere via wet and dry deposition (Haynes *et al.*, 1986).

New Zealand may experience deposition of NH_3 compounds by dry deposition, however, the significance of N oxide deposition of is low due to its geographic isolation from industrial centres (Vitousek *et al.*, 1997).

2.2.2 Transformations

Mineralization

Mineralization is the conversion of organic N from organic material in or on the soil to plant available NH_4^+-N.

Micro-organisms excrete exocellular enzymes onto the organic material, proteinases break down complex proteins into amino acids. Amino acids are then further converted to NH_3 in a sub process called ammonification that the micro-organisms derive energy from:

$$\underset{(Amino\ N)}{R - NH_2} + H_2O \rightarrow NH_3 + R - OH + energy \quad \textbf{(4)}$$

Ammonia is quickly hydrolysed to NH_4^+-N in the soil ready for plant uptake or nitrification (Haynes *et al.*, 1986; McLaren and Cameron, 1996).

Immobilization

Micro-organisms carry out mineralization to generate inorganic-N for their own use. They take up some of the inorganic-N produced and convert it to organic N by incorporating it into their bodies, a process called immobilization.

Micro-organisms need a certain amount of N relative to C. Carbon is always present in material in far greater quantities than N, however, the C:N ratio can be highly variable depending on the N content of the material. Material with a high N content e.g. Lucerne (3% N) has a low C:N ratio (13:1), while Wheat straw has a low N content (0.5% N) and a high C:N ratio (80:1) Table 2-2.

Table 2-2 Approximate composition of organic-C, total N and the C:N ratios of various plant and soil materials, from McLaren and Cameron (1996).

Organic material	Organic-C	Total N (%)	C:N ratio (%)
Lucerne (young)	40	3	13:1
Clover (mature)	40	2	20:1
Wheat straw	40	0.5	80:1
Soil humus	2	0.2	10:1
Soil bacteria	50	10	5:1
Soil actinomycetes	50	8.5	6:1
Soil fungi	50	5	10:1

When the C:N ratio is < 25:1, more N is mineralized than the micro-organisms require. Net mineralization occurs and the surplus NH_4^+-N is released into the soil. However, if the material has a C:N ratio > 25:1 there is insufficient N for the micro-organisms requirements. Inorganic-N already present within the soil will be immobilized, leading to net immobilization (McLaren and Cameron, 1996).

Nitrification & Denitrification

Ammonium that is not taken up by plants, fixed by clay minerals or bound by cation exchange can be oxidised to Nitrite (NO_2^--N) by *Nitrosomonas* and further oxidised to NO_3^--N by *Nitrobacter* in the process of *nitrification* (See Section 2.3.3) (Ferguson *et al.*, 2007).

Denitrification (See Section 2.3.3) is an anaerobic process where bacteria can use NO_3^--N as an electron acceptor to oxidise carbohydrates in O_2 limiting conditions. NO_3^--N is reduced, producing in turn, NO_2^--N, NO, N_2O and lastly N_2 if the N hasn't already been lost from the system (McLaren and Cameron, 1996).

2.2.3 Losses

Volatilisation

Volatilisation is the emission of gaseous NH_3 from the soil into the atmosphere. Volatilisation can occur when there is free NH_3 on or near the soil surface (Haynes *et al.*, 1986). Such NH_3 can come from N fertilisers, urine or other organic compounds.

According to the general equation:

$$\underset{(urea)}{CO(NH_2)_2} + 2H_2O \rightarrow \underset{(ammonium\ carbonate)}{(NH_4)_2CO_3} \rightarrow NH_4^+ + NH_3 \uparrow + CO_2 + OH^- \quad \textbf{(5)}$$

Urea from the N source is quickly hydrolysed to ammonium carbonate in moist soil conditions. The Ammonium carbonate dissociates to NH_4^+-N and NH_3 gas, which can volatilize.

Ammonia production is favoured under conditions of high temperature and high pH. The OH^- ions released by the ammonium carbonate raise the pH of the soil, exacerbating NH_3 losses (McLaren and

Cameron, 1996). Anywhere from 0-65% of the N applied can be volatilised from Urea fertilizer depending on soil and environmental conditions (Cameron *et al.*, 2013).

Plant Uptake

Plants can assimilate N in the form of NH_4^+-N and NO_3^--N , although some plants e.g. Wheat, have a strong preference for NO_3^--N (Haynes *et al.*, 1986).

Some crops utilize higher levels of N than others e.g. an 18 t ha^{-1} Lucerne crop will utilise 500 kg N ha^{-1} compared with a 11.5 t ha^{-1} wheat crop, which only uses 155 kg N ha^{-1} (Table 2-3).

Table 2-3 Amounts of N taken up by various agricultural crops (considerable variations possible depending on soil/fertilizer N supply), from McLaren and Cameron (1996).

Crop		Uptake (kg N ha^{-1})
Wheat	(Grain: 5.5 t ha^{-1})	110
Wheat	(Straw; 6.0 t ha^{-1})	45
Grass	(10 t ha^{-1})	300
Lucerne	(18 t ha^{-1})	500
Potatoes	(Tubers; 55 t ha^{-1})	170
Potatoes	(Vines; 5 t ha^{-1})	115
Maize	(19 t ha^{-1})	330
Rice	(Grain; 8 t ha^{-1})	85
Rice	(Straw; 10 t ha^{-1})	40

Plants with lower levels of production *generally* use less N. A plant's uptake also depends on a number of soil and environmental factors including N availability and suitability of the climate for plant growth (Haynes *et al.*, 1986).

Nitrate leaching

The two forms of inorganic-N in soil have opposite charge. NH_4^+-N is positively charged, therefore, in addition to being immobilized by soil micro-organisms and fixed by clay minerals it is also attracted by the negatively charged cation exchange sites of soil colloids. In contrast, NO_3^--N is negatively charged and so it is repelled by the negatively charged cation exchange sites, and easily leached when water moves through the soil profile (McLaren and Cameron, 1996).

This is not only an economic loss, through reduction in soil fertility, but also an environmental issue as excess N in ground and surface waters can render the water unpotable and contribute to eutrophication of streams, rivers and lakes (Cameron *et al.*, 2013).

The quantity of water moving through the soil profile and the concentration of NO_3^--N in the soil solution determine the leaching losses. The concentration of NO_3^--N in solution is largely influenced by N inputs to the soil and the processes, which trigger mineralization and nitrification e.g. cultivation (Haynes *et al.*, 1986).

Dymond *et al.* (2013) observed that NO_3^--N leaching is strongly correlated with the intensity of agriculture. They estimated that NO_3^--N leaching in Canterbury doubled from 10 million kg N yr^{-1} in 1990 to 20 million kg N yr^{-1} in 2011, associated with a 10-fold increase in dairy cow numbers.

In New Zealand, NO_3^--N leaching losses are greatest between late autumn and early spring. During this period NO_3^--N accumulates in the system due to low plant uptake; soil is near or at field capacity and rainfall exceeds evapotranspiration encouraging drainage and associated leaching (McLaren and Cameron, 1996).

2.3 Nitrous Oxide (N_2O) Emissions

2.3.1 Significance of N_2O emissions

Environmental – impacts on the atmosphere

Nitrous oxide (N_2O) is a potent GHG, having about 12 times the global warming potential of methane and nearly 300 times that of CO_2 over 100 years (Butterbach-Bahl *et al.*, 2013). It is the third most important GHG, contributing to over 6% of the worlds radiative forcing. N_2O (predominately from agricultural soils) contributes 11.2% of New Zealand's GHG emissions. Also of significance is the fact N_2O is the single most important substance responsible for destruction of stratospheric O_3 (Butterbach-Bahl *et al.*, 2013; Ministry for the Environment, 2016; Edenhofer *et al.*, 2014; Ravishankara *et al.*, 2009).

Only c.a. 10% of N_2O emitted to the atmosphere is responsible for the destruction of N_2O. This is the portion of N_2O that is converted to NO in the stratosphere (See Section 2.2.1). NO_X (which includes NO converted from N_2O) in the stratosphere above c.a. 25km reacts with and destroys O_3. Direct emissions of NO_X from the earth's surface are not expected to travel up in to the stratosphere and contribute to O_3 destruction due to their short atmospheric life (Haynes *et al.*, 1986). Nitrogen oxides react with O and O_3 to produce O_2 and the opposite N oxide according to the equation:

$$NO + O_3 \rightarrow NO_2 + O_2 \quad \textbf{(6)}$$

$$O + NO_2 \rightarrow NO + O_2 \quad \textbf{(7)}$$

$$Net: O + O_3 \rightarrow 2O_2 \quad \textbf{(8)}$$

Therefore, when both NO and NO_2 (NOx) are present in the stratosphere the pair can repeatedly destroy stratospheric O_3 (Johnston, 1971).

It has long been known that tropospheric concentration of N_2O was increasing, however, it wasn't until the 1970's that atmospheric scientists discovered its detrimental effects in the atmosphere (Bremner, 1997). This has triggered significant scientific research in the last four decades, although

understanding of the processes involved in N_2O production is still very limited (Butterbach-Bahl *et al.*, 2013).

Economic – Nitrogen loss from agricultural soils

Greenhouse gas emissions from agriculture were 39,585.3 kt CO_2-e in 2014. Nitrous oxide emissions comprised 21.5 % of all agricultural emissions (8510.8 kt CO_2-e) in New Zealand in this year (Ministry for the Environment, 2016).

The calculation below details the economic loss resulting from N_2O emissions from agriculture, based on the level of N_2O emissions from agriculture quantified by the Ministry for the Environment (2016) and the cost of urea fertiliser at \$482 t^{-1}, to replace the N (Ravensdown, 2017).

Converting CO_2 equivalents to mass of N_2O emitted

8510.8 kt CO_2-e ÷ 298 (global warming potential of N_2O) = 28.6 kt N_2O.

Calculating the mass of N lost

Molecular mass ratio of N/N_2O = 28/44.

28/44 × 28.6 kt N_2O = 18.2kt N.

18.2 kt N = 18,200 t N.

Cost of replacing the N with urea fertiliser (46 % N)

18,200 t ÷ 46 % N × \$482 t^{-1} = 19,000,000

\$19 million of urea (excluding GST and spreading etc.) would be required to replace N lost from agricultural soils in NZ in 2014.

2.3.2 Processes producing N_2O

Nitrous oxide can be produced from a large number of biotic and abiotic pathways (Heil *et al.*, 2016; Stevens and Laughlin, 1998). Historically N_2O was believed to evolve solely from denitrification (Bremner, 1997), however, an increasing number of pathways are being proposed with conflicting evidence and suggestions of their relative importance to total N_2O emissions (Kampschreur *et al.*, 2011; Venterea, 2007). It is now well established that nitrification can produce N_2O, arguably of greater quantities than denitrification (Bremner, 1997; Khalil *et al.*, 2004). Never the less, nitrification and denitrification are currently assumed to be the two major sources of N_2O in agricultural soils (Bremner, 1997; Butterbach-Bahl *et al.*, 2013; Kool *et al.*, 2009). Before pathways of N_2O evolution can be understood it is important to understand the general process of nitrification and denitrification.

Under aerobic conditions nitrifying bacteria use the energy released from the oxidation of NH_4^+-N or NO_2^--N for cell growth. Carbon for cell constituents is derived from reduction of CO_2 in the soil

matrix, driven by ATP formed during oxidation of NH_4^+-N or intermediate NO_2^--N (Ferguson *et al.*, 2007; Haynes *et al.*, 1986).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + energy \quad \textbf{(9)}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + energy \quad \textbf{(10)}$$

Recently, a complete ammonia oxidising bacteria ("comammox"), that can oxidise both NH_3 to NO_2^--N and NO_2^--N to NO_3^--N, was isolated. It is of the genus *Nitrospira* and has been provisionally termed *Candidatus* Nitrospira inopinata (*Ca.* N. inopinata) (Daims *et al.*, 2015). However, until this time nitrification has been long accepted as requiring both ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB) to nitrify NH_3 to NO_3^--N. AOB such as *Nitrosomonas* oxidise NH_4^+-N to NO_2^--N. *Nitrosomonas* is the best studied but not necessarily the most common AOB (Arp and Stein, 2003; Chain *et al.*, 2003). *Nitrobacter* is believed to be the most important NOB, oxidising NO_2^--N to NO_3^--N (Ferguson *et al.*, 2007).

The NH_3 oxidation by AOB is a two stage process. Firstly, ammonia monooxygenase catalyses the reaction of NH_4^+-N to hydroxylamine (NH_2OH), using one atom of O_2 and two electrons. Secondly, hydroxylamine dehydrogenase oxidises NH_2OH to NO_2^--N (Ferguson *et al.*, 2007). Nitrite rarely accumulates in the soil as its oxidation to NO_3^--N by NOB is more rapid than its conversion from NH_4^+-N (Heil *et al.*, 2016).

Denitrification is the bacterial reduction of NO_3^--N to N_2 with NO and N_2O as obligate intermediates or end products if incomplete denitrification occurs. Bacteria facilitate the reaction in anaerobic conditions where NO_3^--N can be used as an electron acceptor (Butterbach-Bahl *et al.*, 2013).

Kool *et al.* (2009) uses the equation detailed in Figure 2-2.

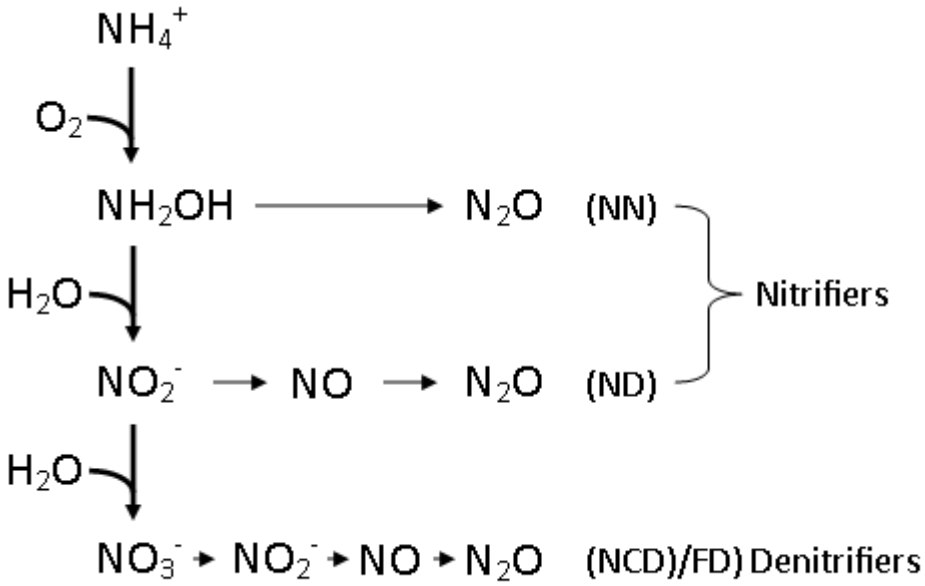

Figure 2-2 Nitrification and associated losses of N_2O. NN = Nitrifier Nitrification, ND = Nitrifier Denitrification, NCD = Nitrifier Coupled Denitrification and FD = Fertiliser Denitrification.

2.3.3 Pathways of N_2O production

(Corbet, 1935) was the first to suggest that N_2O could be produced through nitrification. Nitrogen was lost from the system and later detected to be N_2O. He proposed the nitrification equation:

$$NH_3 \rightarrow NH_2OH \rightarrow H_2N_2O_2 \rightarrow HNO_2 \rightarrow HNO_3 \quad \textbf{(11)}$$

And that N_2O was being produced from the dissociation of hyponitrous acid ($H_2N_2O_2 = H_2O + N_2O$) Kool *et al.* (2009) terms this loss of N as nitrifier nitrification. Initially N_2O production by NH_2OH was deemed a biological process as NH_2OH was rarely detected in, an assumed not to be released in the soil (Arp and Stein, 2003; Bremner *et al.*, 1980; Heil *et al.*, 2016; Spott and Stange, 2011). Moews Jr and Audrieth (1959) had earlier reported that NH_2OH will not usually accumulate in soil because of its very reactive nature. Bremner *et al.* (1980) concluded that NH_2OH may actually be released into the soil, however, its rapid oxidation to NO_2^--N or decomposition to N_2O may prevent its detection. (Liu *et al.*, 2014) supported this claim, extremely precise equipment was used to detect hydroxylamine's presence in soil. The rapid rate of N_2O produced when NH_2OH is added to soil (Figure 2-4) builds on previous speculation that the evolution of N_2O from soil in the initial phase of NH_3 oxidation is the chemical decomposition of NH_2OH, potentially producing more N_2O than nitrifier denitrification (Spott and Stange, 2011). Biological reactions from NH_2OH would be unlikely to produce N_2O as rapidly as illustrated in Figure 2-3 where sterile soil was used.

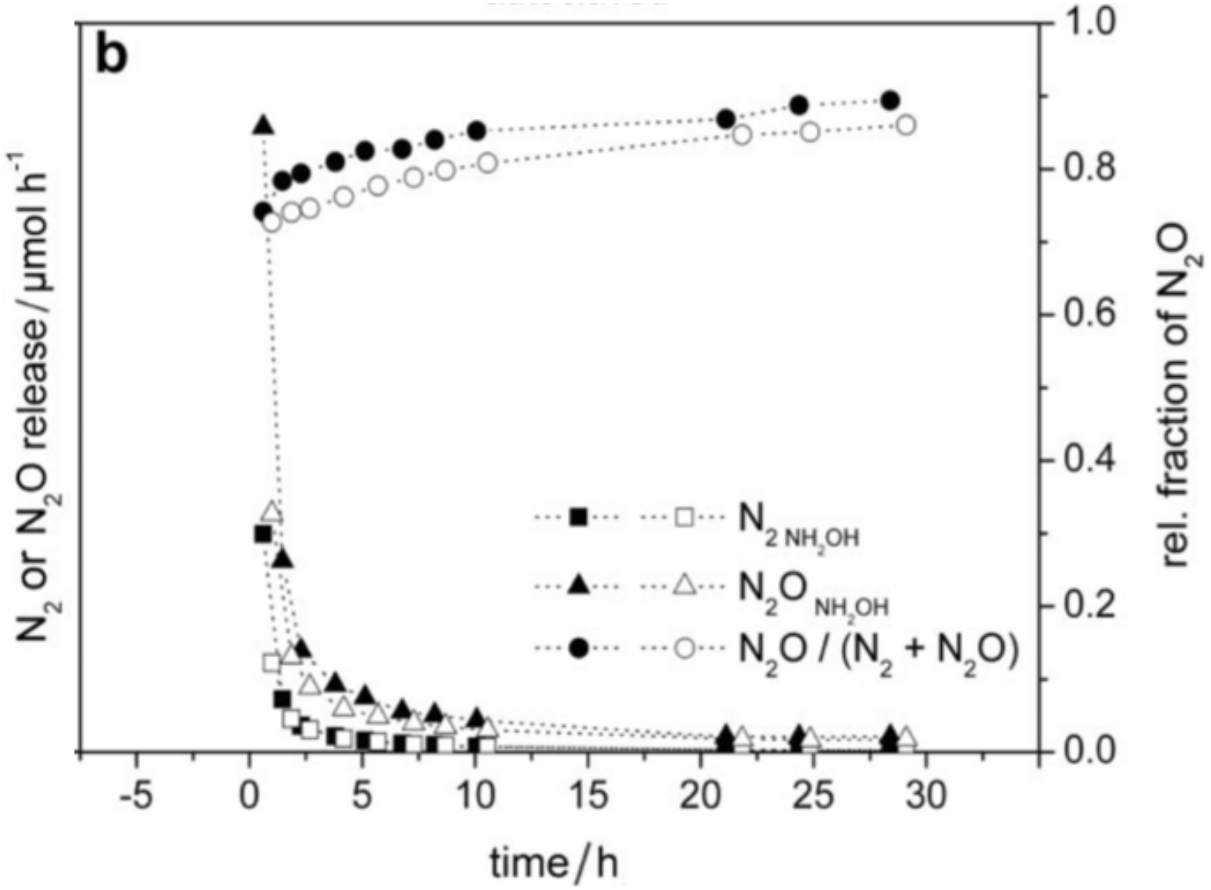

Figure 2-3 N_2 and N_2O production based on $^{15}NH_2OH$ conversion and relative fraction of N_2O on total N-N-gas production using an autoclaved soil suspension (black and white symbols refer to replicate 1 and 2, respectively; time = 0 represents NH_2OH application) from Spott and Stange (2011).

In O_2 limiting environments, < 5% O_2, AOB can use NO_2^--N as an electron acceptor for NH_3 oxidation, reducing NO_2^--N through nitrifier denitrification to NO then N_2O (Firestone *et al.*, 1980; Khalil *et al.*, 2004; Poth and Focht, 1985; Ritchie and Nicholas, 1972). Wrage *et al.* (2001) have suggested that nitrifier denitrification can be an important source of N_2O emissions in circumstances of a low C:N ratio, low O_2 pressure and potentially low pH. The conversion of N_2O to N_2 is currently only attributed to the autotrophic nitrifier – *Nitrosomonas sp (Zhu et al., 2013).*

N_2 is not produced as a product of nitrifier denitrification as nitrifiers do not have the nitrous oxide reductase gene to reduce N_2O to N_2 (Ferguson *et al.*, 2007). Nitrifier denitrification allows nitrifiers to conserve limited O_2 for the initial fixed step of NH_3 oxidation, remove competition for O_2 by consuming the substrate for NO_2^--N oxidisers and prevent toxic concentrations of NO_2^--N accumulating (Bremner, 1997; Ferguson *et al.*, 2007; Poth and Focht, 1985).

Denitrification produces N_2O (as described earlier) when the reaction is incomplete. NO emissions are rare as it rarely accumulates, due to rapid reduction to N_2O. However, N_2O often diffuses out of the soil before it is reduced to N_2. It appears that some bacteria do not have the N_2O reductase enzyme, hence the process ends at N_2O when such bacteria denitrify (Firestone, 1982; Stevens and Laughlin, 1998).

Nitrifier coupled denitrification (NCD) describes the use of NO_3^--N by denitrifiers, when in close proximity to NO_3^--N of nitrification origin (Kool *et al.*, 2009). Fertilizer denitrification characterises the denitrification of fertilizer NO_3^--N (Butterbach-Bahl *et al.*, 2013).

Co-denitrification accompanies denitrification where NO_3^--N, in conjunction with other N compounds, forms N_2O. It can yield 2 mols of N_2O per 2 mols of NO_3^--N compared with denitrification's 1 mol of N_2O per 2 mols of NO_3^--N, however, its significance in agricultural soils is questionable (Butterbach-Bahl *et al.*, 2013; Spott and Stange, 2011).

Dissimilatory reduction of NO_3^--N to ammonium (DNRA) is an alternative pathway to denitrification for NO_3^--N / NO_2^--N.

$$NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+ \quad \textbf{(12)}$$

Nitrate is reduced to NO_2^--N, it is then possible for some NO_2^--N to be reduced directly to N_2O instead of NH_4^+-N (Paul & Beauchamp, 1989; Stevens & Laughlin, 1998) (Paul and Beauchamp, 1989; Stevens and Laughlin, 1998).

Chemo denitrification is the production of N_2O associated with chemical decomposition of NO_2^--N (Heil *et al.*, 2015).

2.3.4 Factors affecting N_2O emissions and mitigation options

Denitrification and nitrification are the major sources of N_2O emissions in agricultural soils, therefore, factors that affect nitrification and denitrification will influence N_2O emissions.

Nitrogen

Additions

Increasing inputs of N to the soil will increase N_2O emissions. Bell *et al.* (2016) recorded that when the application rate of ammonium nitrate increased from 80kg N ha^{-1} to 400 kg N ha^{-1} the EF increased 64% from 1.06% to 1.74%, respectively.

Similarly, Figure 2-4 shows that large applications of NO_3^--N e.g. sodium nitrate, that increase the concentration of NO_3^--N and especially NO_2^--N, can favour N_2O production as opposed to N_2 production.

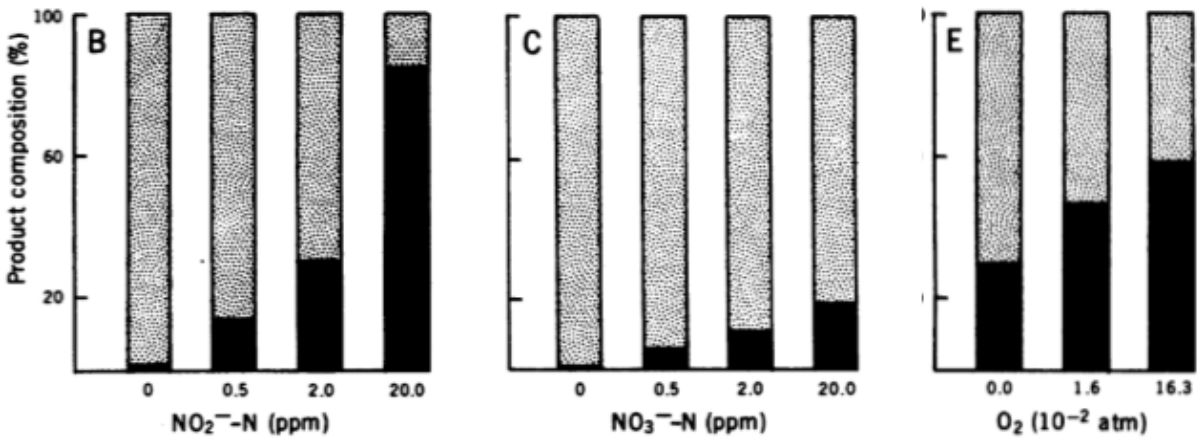

Figure 2-4 Composition of the gaseous products of denitrification in a Brookston soil. The black bars indicate N_2O and the dotted areas represent N_2. Effect of NO_2^- (B), NO_3^- (C) and O_2 (E) concentration on N_2O and N_2 production, from Firestone *et al.* (1980).

In support of increasing N rates' influence on N_2O emissions, Roy *et al.* (2014) reported a reduction in cumulative emissions of a corn crop from 2.604 kg N ha^{-1} when 218 kg N ha^{-1} was applied (E.F. 1.19%) to 0.318 kg N ha^{-1} when the rate applied dropped to 30 kg N ha^{-1} (E.F. 1.06%). However, it was noted that WFPS had a much greater effect on N_2O emissions.

The use of minimum or no tillage in favour of conventional cultivation normally reduces N_2O emissions. Less disturbance of the soil decreases mineralization of organic N, reducing the availability of NH_3 as a substrate for N_2O production. García-Marco *et al.* (2016) observed that tillage increased N_2O emissions 68% compared with non-tillage.

Slow release fertilisers and nitrification inhibitors also reduce N_2O emissions through minimising surplus N by better matching plant demand. A meta-analysis by (Akiyama *et al.*, 2010) of 35 studies found slow release fertilizers and nitrification inhibitors reduced N_2O losses on average by 35% and 38%, respectively in comparison to conventional fertilizer. Slow release fertilisers use a semi permeable coating that restricts the diffusion of N, slowing its release. Nitrification inhibitors minimise nitrification and associated N losses by inhibiting the ammonia mono-oxygenase enzyme, blocking the oxidation of NH_3 to NO_2^--N until the primary crop is at its log phase of growth. Reducing the production of NO_2^--N and NO_3^--N also reduces losses of N_2O through denitrification.

Further options to minimise N input to the system include reducing the N content of plants to better batch the needs of animals (Haynes *et al.*, 1986) and maximising the N use efficiency (NUE) of both plants and animals. New rice cultivars with a higher NUE have not only been observed to yield higher from the same N as applied to conventional cultivars, but also on a yield scaled basis they have reduced N_2O emissions by 23-26% (Chen *et al.*, 2015).

Form of N applied

Form of N is also important. After spring and early summer fertiliser applications, to an intensively managed pasture, cumulative N_2O fluxes after one month were greatest in plots treated with ammonium nitrate (5.2 kg N ha^{-1}), significantly higher than those treated with urea (1.4kg N/ha) (Dobbie and Smith, 2003). Authors cited greater losses from denitrification in the ammonium nitrate treated plots due to higher NO_3^--N concentrations.

Toxicities

Venterea (2007) indicated that when NH_3 is present in high concentrations it is more toxic to NO_2^--N oxidising bacteria than NH_3 oxidising bacteria leading to accumulations of NO_2^--N in the soil promoting NO_2^--N driven N_2O production in the nitrification phase. This suggests that not only will large applications of NH_3 to soil produce more N_2O, they will produce relatively more N_2O emissions than several smaller applications.

Timing of N

Delaying effluent after grazing avoids surplus inorganic-N from double ups of excreta and FDE. Luo *et al.* (2008b) observed a significant increase in N_2O emissions from 0.449 to 0.004 kg N ha^{-1} when FDE application was delayed 2 months after grazing. Delays of 10-20 days after grazing may be possible in practice, however, depending on the time of year, delays of two months would only be possible in paddocks used for cut and carry. Never the less, Li *et al.* (2016) are in agreement with delaying FDE application after grazing, reporting a strong interaction from FDE applied to urine patches. Addition of FDE to a urine patch increased the N_2O EF (% of applied N emitted as N_2O) for the urine from 1.02 to 1.40%.

Soil Moisture & Aeration

Oxygen concentration and soil moisture content, a driver of O_2 concentration, have the greatest influence on nitrification and denitrification. N_2O emissions increase with water-filled pore space (WFPS) (Figure 2-5). Large increases in N_2O emissions are observed when WFPS exceeds 60% (Clough *et al.*, 2004).

It is well known that nitrification is an aerobic process and denitrification an anaerobic process (Bremner, 1997). Increased WFPS reduces the aeration of the soil, promoting denitrification. Large N_2O fluxes produced at high WFPS as illustrated in Figure 2-5 support the conclusion of Firestone and Davidson (1989) that large N_2O fluxes are normally associated with denitrification rather than nitrification.

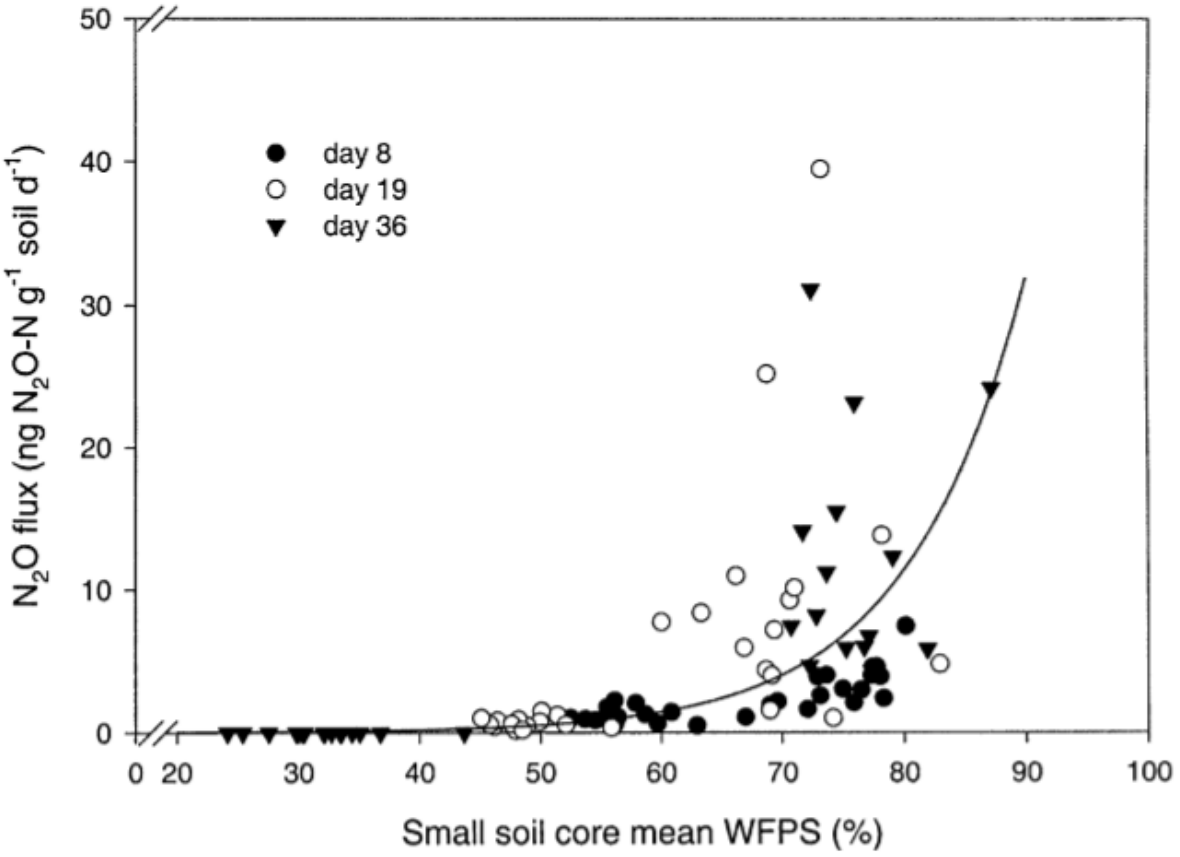

Figure 2-5 Effect of water-filled pore space (WFPS) on the N_2O-N flux at three times following urine application, during small soil core destructive analysis. Water-filled pore space is the average of the entire soil core. Data points represent individual replicates. Fitted line is an exponential regression [y = 0.003exp(0.103x), r2 = 0.8] for all data points, from Clough *et al.* (2004).

Further in support, Maag and Vinther (1996) reported a sharp increase in N_2O emissions when WFPS increased from 40% to 100% of FC. N_2O produced by nitrification at 10°C (as a percentage of NO_3^--N produced) increased from 0.25 to 0.89%. Dobbie and Smith (2001) observed N_2O emissions increased 12 – 30 fold when WFPS increased from 60 to 80%.

Firestone *et al.* (1980) found that O_2 concentration influenced both the distribution as well as the quantity of gaseous products of denitrification (Figure 2-4E) When anaerobic conditions were imposed N_2O emissions initially increased and comprised half of all gaseous losses in the first 12 hours then declined rapidly relative to N_2 from 23 hours onwards. This decline in N_2O emissions is thought to coincide with synthesis of the N_2O reductase enzyme leading to N_2O consumption exceeding production. The concentration of liquid O_2 is the determining factor of a soils aeration. The diffusion of O_2 in water is 105 times slower in water than air (Poth and Focht, 1985).

At O_2 concentrations less than 0.35 kPa nitrification was the main source of N_2O emissions (Khalil *et al.*, 2004).

Carbon

Chemoautotrophs that carry out nitrification obtain their C through assimilation of CO_2, however, heterotrophic denitrifiers require C for their cellular material and as an energy donor. Therefore, the availability of C, specifically readily available organic-C is an important factor determining the rate of denitrification and N_2O emission. Inputs of organic material will increase the rate of denitrification and increase emissions. However, there may be a threshold C level, as C availability allows for the full denitrification of NO_3^--N past N_2O to N_2, decreasing the N_2O:N_2 ratio (Haynes *et al.*, 1986). Soils with higher organic matter often exert higher N_2O fluxes (Stehfest and Bouwman, 2006). Conventional tillage practices conducive to mineralization can degrade the level of organic matter over time, reducing the potential for denitrification, however, this is not desirable in terms of sustaining soil structure (Saggar *et al.*, 2004).

Inputs to soil with a high C:N ratio (See Section 2.2.2) e.g. Lucerne will result in net mineralization, supplying a greater level of NH_4^+-N for the production of N_2O (Haynes *et al.*, 1986).

Temperature

Temperature determines the level of bacterial activity and hence the level of nitrification and denitrification. Increases in temperature increase the rate of nitrification and denitrification, however, the rate of N_2O-N produced relative to other N products decreases (Maag and Vinther, 1996). Higher temperatures can also decrease the solubility and increase the rate of diffusion of N_2O in water leading to higher emissions. An increase in the temperature from 10 to 40°C produced a 10-fold increase in N_2O emissions when the soil was treated with 150 mg N kg^{-1} as urea (Cai *et al.*, 2016). Goodroad and Keeney (1984) had also reported an increase in N_2O emissions from 13 to 33 ng N_2O g^{-1} soil when the temperature increased from 10 to 30°C and noted diurnal fluctuations of N_2O following the daily temperature pattern.

Soil pH

Soil N_2O emissions are strongly associated with soil pH (Mørkved *et al.*, 2007; Qu *et al.*, 2014). The optimum pH for both nitrifying and denitrifying bacteria is above 7, therefore, it would be expected that N_2O emissions would rise as pH rose towards this optimum (Haynes *et al.*, 1986). However, bacterial inhibition does not appear to be the main driver of this pH effect. Although the rate of denitrification is greater at high pH, N_2O production and the $N_2O/(N_2O+N_2)$ product ratio both decrease as pH increases (Firestone and Davidson, 1989; Qu *et al.*, 2014; Raut *et al.*, 2012). Samad *et al.* (2016) observed that N_2O emissions and the $N_2O/(N_2O+N_2)$ product ratio were strongly and negatively associated with soil pH (R^2 = 0.71 to 0.85 and R^2 = 0.68 to 0.82, respectively, depending on the method used to determine the pH). This is consistent with results reported by Bremner *et al.* (1980), concluding that the relative composition of the gaseous products was affected by pH. Soils of

pH 7.8 and above produced 70% more N_2 than N_2O, in contrast soils of pH lower than 7 favoured N_2O production. N_2 production contributed less than 7% to overall N_2-N_2O in the lower pH soils.

A higher $N_2O/(N_2O+N_2)$ product ratio at low pH suggests that denitrification does not proceed to N_2. This is explained by inhibition of the N_2O reductase enzyme at low pH. Bergaust *et al.* (2010) reported that reduction of N_2O was strongly reduced at low pH due to the inhibition of the assembly of N_2O reductase. The action of existing N_2O reductase enzymes were unaffected. Despite an increase in the production of N_2O and an increase in the $N_2O/(N_2O+N_2)$ product ratio at lower pH, reduced rates of denitrification at lower pH (Firestone and Davidson, 1989; Qu *et al.*, 2014; Raut *et al.*, 2012) suggest that total losses of N from denitrification will be reduced at lower pH. In this regard, economic and environmental optimums are not aligned.

Liming soils to above pH 7 may significantly favour N_2 production, however, it may substantially increase N_2 (a loss of N from the soil) without decreasing N_2O volumes. Similarly, raising the pH to such a level is not recommended. Not only would this be very costly and uneconomic, it would also reduce the availability of some essential nutrients in the soil and induce toxicities of others. Raising the pH of acidic soils to approximately pH 6 is more appropriate when considering the overall soil/plant system pH requirements.

Clough *et al.* (2004) reported that liming to pH 5.8 has merit to reduce N_2O emissions when soils are at field capacity (Figure 2-6), however, N_2O emissions are positively correlated with pH when soils are saturated.

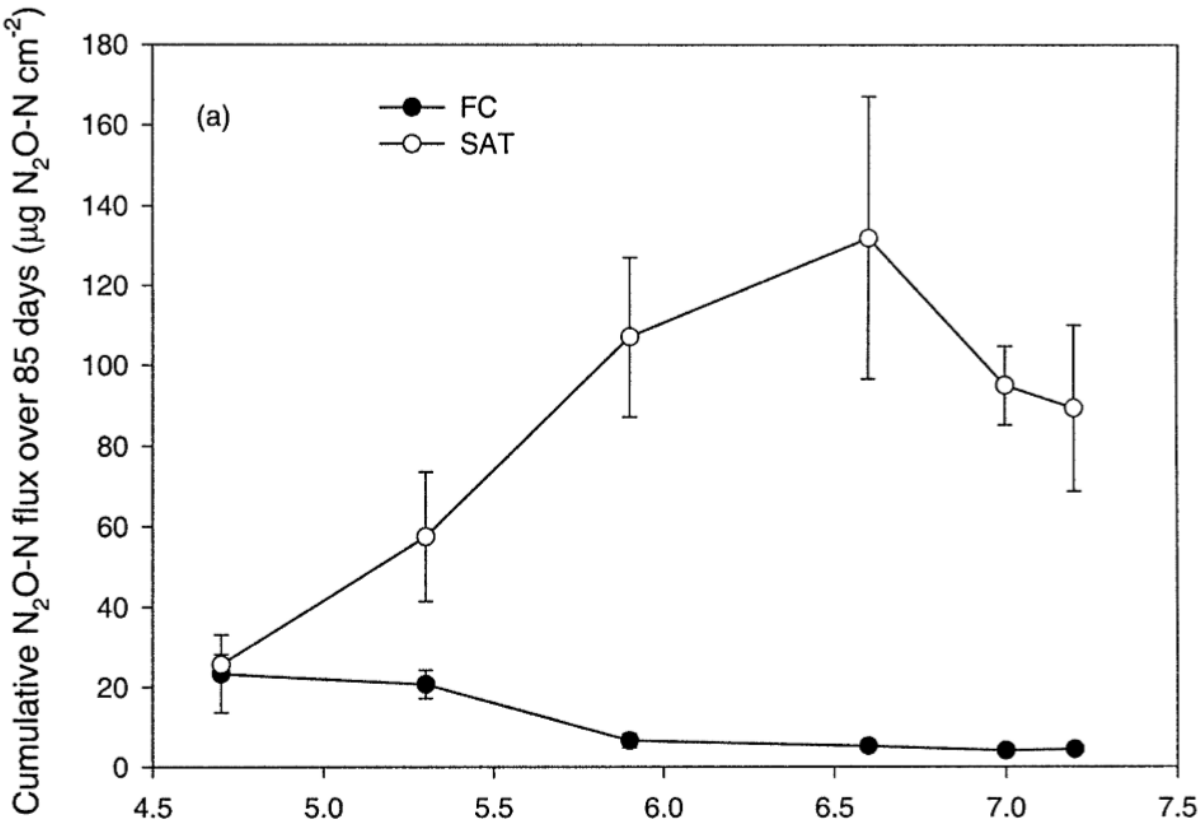

Figure 2-6 Cumulative N_2O-N flux after 85 days versus the initial soil pH of soils at saturation (SAT) or field capacity (FC), from Clough *et al.* (2004).

A recent experiment by García-Marco *et al.* (2016) that aimed to analyse the effects of liming in both conventionally tilled (CT) and non-tilled (NT) treatments also produced mixed results. Liming to raise the pH from 5.3 to 6.2 decreased N_2O emissions by 61% in the CT treatment but had no effect on the NT treatment. The effect of liming is still poorly understood, microbial processes especially the dominance of nitrification or denitrification is likely to play a large role in how N_2O emissions respond.

Schmidt *et al.* (2011) found that DNRA (a sink of N_2O) was favoured at elevated pH of above 7, reducing emissions, however, again it is not practical to rise the pH of soils to this level.

McMillan *et al.* (2016) observed that under anaerobic conditions the $N_2O/(N_2O+N_2)$ product ratio decreased with an addition of lime to a weakly buffered fluvial soil but not a well buffered volcanic soil. This indicated that liming may have enhanced the activity of the N_2O reductase enzyme in the fluvial soil but not the volcanic soil. Authors concluded that a soil type-pH interaction due to the allophanic material present in the volcanic soil was the cause of reduced N_2O consumption. Allophane absorbs copper, especially as pH increases, reducing the availability of copper and limiting the activity of N_2O-R.

In summary, it appears that liming acidic soils will reduce N_2O emissions, however, other factors such as soil type can affect the significance of the pH-N_2O emission relationship.

Although not a pH effect, Gao *et al.* (2016) has found that inoculating N_2O reducing denitrifier strains into the soil can reduce N_2O emissions, indicating that such strains are lacking in some soils, leading to greater N_2O emissions.

2.4 N Inputs

Agriculture is the largest source of greenhouse gas emissions in New Zealand, contributing 48% in 2014. Emissions from agricultural soils contribute 8526.3 kt CO_2-e (11%) to New Zealand's emissions profile and 94% of the country's total N_2O emissions (Ministry for the Environment, 2016). Emissions from the agricultural soils category largely result from additions of urine and dung deposited by grazing animals, synthetic N fertiliser and land application of animal wastes (Table 2-4).

Table 2-4 Trends and relative contributions of the top three nitrous oxide emitting Agricultural subcategories between 1990 and 2014, adapted from Ministry for the Environment (2016).

	Emissions (kt CO_2-e)		Change from 1990	Share of total Agricultural sector
	1990	2014	%	2014 %
Urine and Dung	5255.5	5713.0	8.7	14.4
Synthetic fertilisers	217.7	948.1	335.5	2.4
Effluent	78.5	153.1	95.0	0.4

Urine and synthetic fertiliser are the most significant contributors of N_2O emissions from agricultural soils in New Zealand, receiving the most attention from researchers as a result. In contrast, the land application of FDE only contributes 0.4% to New Zealand's total GHG emissions, therefore, less focus has been placed on minimising emissions from this N input.

Dairy farming has intensified rapidly since 1990. Cow numbers have doubled and synthetic N fertiliser use has increased 5 fold. This is the main factor contributing to the increases in emissions since 1990 as shown in Table 2-4 (Ministry for the Environment, 2016).

N application rate on a dairy farm is variable. Where-as fertilisers are applied according to plant needs and regulatory constraints, farmers often apply FDE year-round including winter, in order to keep their FDE pond at a manageable level. Urine patches are poorly distributed over the paddock and contain extremely high concentrations of N, up to 1000 kg N ha^{-1} (Di and Cameron, 2002b), far above plant needs (Haynes *et al.*, 1986). Variability of the physical and chemical characteristics of FDE (See Section 2.5.3) makes it difficult to determine average N inputs from FDE on a typical dairy farm, however, Van der Weerden *et al.* (2016a) found that total N applied in previous FDE field trials

ranged from 13 to 101 kg N ha^{-1}. Although it is difficult to confirm whether such applications are representative of typical dairy farms. Several applications of N fertiliser as urea (up to 200 kg N ha^{-1} $year^{-1}$) are applied on dairy farms at rates of 30-50 kg N ha^{-1}, mostly in spring (van der Weerden *et al.*, 2016b).

New Zealand currently uses 0.48% and 1% as the EF for urea and FDE, respectively, to calculate its national N_2O inventory. Urine also has an EF of 1%. However, a recent meta-analysis of previous urea and FDE studies by Van der Weerden *et al.* (2016a) has concluded that the values for urea and FDE should be updated to 0.6% and 0.3%, respectively. The EF of 0.3% for FDE was encompassed in the review of the GHG emissions from FDE by Laubach *et al.* (2015), which reported a range in the EF's of 0.01% to 1.2% from effluent total N concentrations of 13 to 100 kg N ha^{-1}. However, a recent study by Li *et al.* (2016) involving FDE produced an EF of 0.62%, acknowledging that N_2O emissions can be highly variable due to a number of factors (See Section 2.3.4).

Di and Cameron (2008) reported a significantly higher EF for urea than the IPCC and Van der Weerden *et al.* (2016a)'s factors of 0.48 and 0.6%, respectively. However, the authors attributed this to a very wet period during the trial, potentially stimulating a lot of N_2O emissions from denitrification. Singh *et al.* (2013) had also observed a much larger EF from urea of 4.27%, however, an atypical application of 100 kg N ha^{-1} of urea was applied. This is much large than normal fertiliser applications so large amounts of substrate may have been available for N_2O production.

A trial to estimate the N_2O EF of cow urine on N pastoral soil concluded that rainfall and drainage class have a strong influence on urines EF. Emission factors ranged from 0.3 – 2.5%, the highest EF was recorded on a poorly drained soil while the lowest was from a well-drained stony site (De Klein *et al.*, 2003). The IPCC EF for urine of 1% (Van der Weerden *et al.*, 2016a) is within this range. Luo *et al.* (2008a) also reported results in agreement with previous findings, recording EF's in the range of 0.02 to 1.52% to for cow urine.

Despite large variations in the (recommended) EF's of urine (1%), urea (0.6%) and FDE (0.3%), the size of the EF relative to each other seems accurate. Urine has the highest EF as it is extremely concentrated, far exceeding plant N requirements, resulting in large amounts of surplus inorganic-N substrate for N_2O production. In addition, urine increases the soil WFPS encouraging (very temporary) denitrification. Urea although highly concentrated in its own right is applied in lower concentrations and lacks liquid, deriving a lower EF. Finally, FDE is a combination of urine, dung and water, so a mixture of unavailable organic N as well as plant available NH_4^+-N and NO_3^-N, in addition, the N is watered down compared to a urine patch, hence its low EF (Van der Weerden *et al.*, 2016a). Despite this, (Li *et al.*, 2016) observed a positive interaction between dung and urine, the

presence of dung doubling the EF of urine from 1.02% to 2.09%, the dung contributed excess C to the urine, which had excess NH_4^+-N but lacked C.

Although the land application of FDE is a minor contributor to New Zealand's overall emissions profile, the mass applied to land increased from 18kt in 1990 to 39kt in 2013 (Van der Weerden *et al.*, 2016a). In addition, increases in popularity of herd homes is likely to see volumes of FDE increase significantly in the future, raising its contribution of N_2O emissions. Therefore, FDE requires further research and therefore, will be the focus of the remainder of the review and subsequent research.

2.5 FDE

2.5.1 FDE and its Influence on N_2O Emissions

FDE is an excreta containing substance with a DM content of less than 5% (Houlbrooke *et al.*, 2011). Section 2.5.3 details further physical and chemical characteristics of typical FDE in New Zealand.

FDE drives N_2O production through addition of N and C as substrates that fuel microbial growth, and by increasing the WFPS, creating anaerobic zones conducive to denitrification (Laubach *et al.*, 2015)

2.5.2 Land Application of FDE in New Zealand

Overview

A doubling of dairy cow numbers since 1990 has significantly increased the volume of FDE applied to pasture from 18kt in 1990 to 39kt in 2013 (Ministry for the Environment, 2016). Intensification of dairy farming has seen a greater use of off-paddock facilities such as herd homes and feed pads, which increase the proportion of cow's excreta represented as FDE and hence increases FDE volumes further (Houlbrooke *et al.*, 2011; van der Weerden *et al.*, 2016b). Saggar *et al.* (2004) estimated 70 million litres of FDE are produced per year in New Zealand, although this is likely to be well out of date.

Science, Regulations & Best Practice

FDE was traditionally discharged to streams and rivers after solids were settled in ponds, however, introduction of the Resource Management Act (RMA) 1991 is seeing this practice phased out by councils, especially in the South Island of New Zealand. Application to land is now the preferred option to discharge FDE (Houlbrooke, 2008; Dairy NZ, 2015).

Current regulations are centred on minimising NO_3^--N leaching, that degrades ground and surface water quality. Little consideration is given for the gaseous emissions such as NH_3 volatilisation and N_2O emission that occur (Laubach *et al.*, 2015).

In Canterbury conditions limiting the annual application of N (including FDE) to 200 kg N ha^{-1} $year^{-1}$ and 100 kg N ha^{-1} are typically placed on discharge consents to limit the impact on water quality (Environment Canterbury Regional Council, 2015). This is a similar condition around the country, although some councils have stricter limits of 150 kg N ha^{-1} $year^{-1}$ or even 75 kg N ha^{-1} $year^{-1}$ in sensitive areas. Farms in extremely sensitive areas may have even tougher, specific consent conditions imposed or simply may be denied consent.

In addition, Environment Canterbury limits the input of N to 100 kg N ha^{-1} in any three-month period.

Some councils enforce maximum FDE application depths (mm) and rates (mm/hr) (Table 2-5) often depending on soil type (Environment Canterbury Regional Council, 2015; West Coast Regional Council, 2015). The remainder of councils imply such restrictions in the consent e.g. "provided: - Effluent does not directly enter any drain, water race or groundwater, and - The discharge does not occur onto saturated soils" (Houlbrooke, 2008; Otago Regional Council, 2004; Taranaki Regional Council, 2016).

Table 2-5 Guidelines for applying effluent, based on soil properties, slope and application tool, from Environment Canterbury Regional Council (2015).

Dairy Effluent (FDE) Risk Categories	A	B	C	D	E
Soil & landscape feature	Artificial drainage or coarse soil structure	Impeded drainage or low infiltration rate	Sloping land (>7°) or land with hump and hollow drainage	Well drained flat land (<7°)	Other well drained but very light flat land (<7°)
Risk	High	High	High	Low	Low
Application depth (mm)	<SWD[1]	<SWD	<SWD	<50% PAW30[2]	<10mm & ≤50% PAW30
Storage requirement	Only apply when SWD exists	Only apply when SWD exists	Only apply when SWD exists	24hours drainage post saturation	24hours drainage post saturation
Max depth: high rate tool[3]	10mm	10mm	10mm[4]	25mm[5] (10mm at field capacity)	10mm
Max depth: low rate tool[6]	25mm	25mm	10mm	25mm	10mm

1 SWD = Soil Water Deficit
2 PAW30 = Plant Available Water in top 30cm of soil
3 A high rate tool is an irrigator that discharges effluent at application rates over 10 millimetres per hour (mm/hr)
4 Only applicable when the instantaneous application rate from the irrigator is less than the infiltration rate.
5 Suggested maximum application depth when a suitable SWD exists (≥15mm)
6 A low rate tool is an irrigator that can discharge at an application rate of less than 10mm/hr.

Note: Application rate refers to the speed (i.e. volume over time), while application depth refers to the depth of effluent and any irrigation water applied to an area over a 24 hour period.

Reviews by (Houlbrooke *et al.*, 2004) and (Houlbrooke *et al.*, 2013) confirm the abundance of science behind the total N loadings, application depths and application rates used by regulatory authorities. Specifically, Roach *et al.* (2001) observed that 200 kg N ha^{-1} $year^{-1}$ did not significantly increase NO_3^--

N leaching above the control, however, it was increased by approximately 150% to approximately 50 kg N ha^{-1} $year^{-1}$ when 400 kg N ha^{-1} was applied (Figure 2-7).

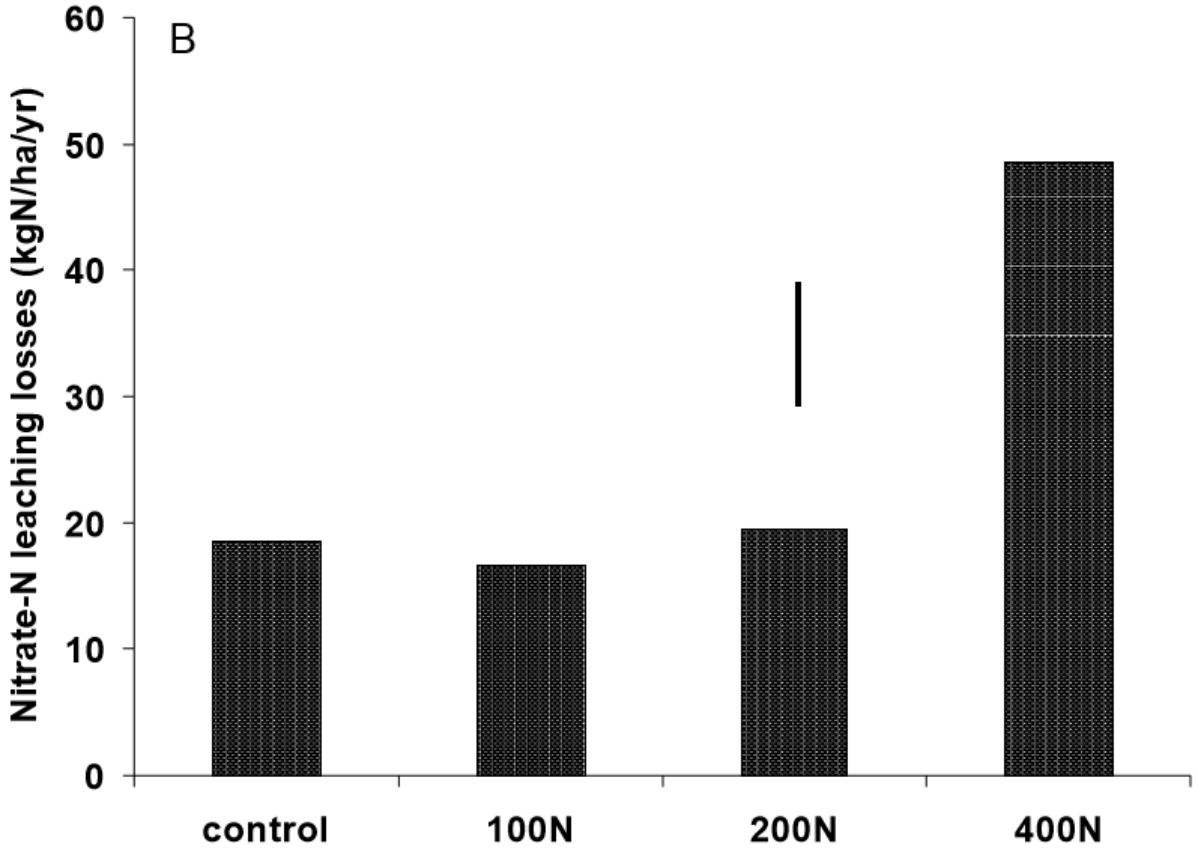

Figure 2-7 Nitrate leaching losses from a three year trial in South Taranaki. FDE was applied monthly from September to April each year in split applications totalling either 0, 100, 200 or 400kg N ha^{-1} $year^{-1}$. Vertical bar is SED. From Roach *et al.* (2001).

Industry organisation, Dairy NZ recommends that less than 50 kg N ha^{-1} is applied per FDE application to maximise pasture uptake and ensure that K application does not exceed maintenance requirements (Logan Bowler, Dairy NZ environment extension specialist, personal communication 26 February 2016).

On Farm

Farmers apply effluent to land using a range of equipment including travelling irrigators, low rate e.g. K-line systems, pivot irrigators or slurry tankers. A range of variables including effluent characteristics, irrigator type, irrigation speed, distance of irrigator from the pump (changes in pressure) and slope of paddock all affect the concentration of N applied making it difficult to determine an accurate value. Values of N applied in studies from the literature ranged from 13 to 101 kg N ha^{-1}. In the past effluent was only applied to a small part of the farm near the effluent pond, however, N loading rates and application depths limit the amount of FDE a farmer can apply to one area. In Otago it is recommended that a farm's effluent discharge area is greater than 8 ha per 100 cows to achieve an approximate application rate of 75 kg N ha^{-1} $year^{-1}$. Farmers are typically increasing the area of their farm that they apply FDE on, by choice, to increase the sustainability of

the FDE application area, which can be compromised my excessive potassium loadings from the application of FDE (Dairy NZ, 2015; Otago Regional Council, 2004; Van der Weerden *et al.*, 2016a). Historically farmers have got into trouble applying FDE to saturated soils because of poor decisions or insufficient FDE storage giving them no option to irrigate. However, effluent storage is now a common requirement by many councils around the country, over 80% of farms had storage ponds in 2015. Canterbury farmers are required to have 15-20 days' storage while farmers in Northland must have ponds large enough to hold 100 days' worth of FDE from their dairy operations (Dairy NZ, 2015; Laubach *et al.*, 2015).

Improved education from leaders in the industry and promotion of best practice has seen the majority of farmers adjust their practices to operate within (or in advance of) tighter regulations, never the less, there are still some farmers who choose not to comply.

2.5.3 Characteristics of FDE

Unlike inorganic-N fertilizers applied to pastures, the physical and chemical characteristics of FDE are highly variable. Longhurst *et al.* (2000) critically reviewed the characteristics of FDE, of which many of their findings are summarised within the following section. In agreement with Wallace and Johnstone (2010) few studies have analysed the characteristics of FDE since the review by Longhurst *et al.* (2000).

Physical and chemical characteristics of FDE can be highly variable. A large number of factors influence these characteristics, such as, time of day, season, point in lactation, breed and age of herd, fertiliser policy, pasture type and quality, type of FDE/sludge separation system, time till application and yard wash-down management. Yard wash-down management often has the largest effect on FDE characteristics due to its dilution effects (Longhurst *et al.*, 2000). Table 2-6, Table 2-7 & Table 2-8 summarise the characteristics of FDE used in recent research.

Table 2-6 Nitrogen concentrations of FDE previously used in field trials.

	Nitrogen component (mg L^{-1})			TAN
Study	Total N	NH_4^+-N	NH_4^+ range	(% Total N)
Silva *et al.* (1999)^	240	61	30-101	25%
Degens *et al.* (2000)	167			
Longhurst *et al.* (2000) (Review)	269	48	13-132	18%
Barton and Schipper (2001)	270	99		37%
Sukias *et al.* (2001)	78	40	25-105	51%
Di and Cameron (2002a)	246	58	15-84	24%
Hawke and Summers (2003)		36		
Bhandral *et al.* (2007) from 2003	252	176		70%
Luo *et al.* (2008b)	98			
Bhandral *et al.* (2010)	96	72	68-75	75%
Houlbrooke *et al.* (2011)	450	200	100-300	44%
van der Weerden *et al.* (2016b)	490	254	145-370	52%
Mean	241	104		43%*
Range	78-490		13-370	18-75%

^Not included in Longhurst *et al.* (2000) review
*Calculated from means of Total N and NH_4^+-N concentrations

The unweighted average total N content of FDE based on 12 studies over a 39-year period from 1977 to 2016 was 241 mg N L^{-1}. The NH_4^+-N content was on average 104 mg N L^{-1}, ranging significantly from only 13 in some trials to as high as 370 in others, comprising on average 43% of the total N (Table 2-6).

van der Weerden *et al.* (2016b) were aiming for an FDE of 500 mg N L^{-1} so they used cow urine to "top up" the FDE to 500 mg N L^{-1} where required. Not only does this overstate the total N content of the FDE, it also raises the NH_4^+-N content as cow urine contains c.a. 80% urea, which rapidly hydrolyses to NH_4^+-N (Haynes *et al.*, 1986). This may also overstate the TAN content (52%) reported by van der Weerden *et al.* (2016b). Never the less, higher TAN contents of 70 and 75% were reported by Bhandral *et al.* in 2007 and 2010, respectively.

Excluding data from van der Weerden *et al.* (2016b), it is still likely the N concentrations of FDE have increased in recent years due to intensification of dairy farming. Houlbrooke *et al.* (2011) reported higher total N and NH_4^+-N concentrations of 450 and 200 mg N L^{-1}, respectively.

Li *et al*. (2016; 2015b; 2014) also recorded higher N concentrations, total N up to 1800 mg N L^{-1} and NH_4^+-N up to 900 mg N L^{-1}, however, data from these three studies wasn't included in Table 2-6 as the FDE was produced by hand to 2% DM, therefore, the N concentrations may not have been accurate and could have skewed the results. Consultants in the dairy industry currently use a total N value of 450 mg N L^{-1} for their calculations (Chris Appleby, Fonterra sustainable farming consultant, personal communication 26 February 2016).

Increasing N concentrations of FDE are to be expected as dairy cow herd numbers have doubled since 1990. Shed and yard area have not increased as much, indicating less water per cow is required when washing the yard, meaning FDE will be less diluted than in the past. If this was the case though, we would also expect to see DM% and total C concentration (Table 2-7) increase, however, the lack of valid data prevents confirmation of this. If anything, the trend in total C concentration is decreasing, so reduced dilution of FDE may not explain increasing N concentrations. N concentrations may also have increased due to the five-fold increase in synthetic N fertiliser use since 1990 (Longhurst *et al.*, 2000; Ministry for the Environment, 2016). Fertilising pastures can raise the N content of the herbage, increasing the surplus N ingested and excreted by dairy cows (Haynes *et al.*, 1986).

The stage that the FDE is sampled is also likely to affect the N content. Li *et al*. (2015b; 2014) found that storage and aerobic treatment of FDE in ponds for four months decreased the N concentration. In the 2014 trial total N and NH_4^+-N decreased from 1150 to 1000 and 455 to 155mg N L^{-1}, respectively.

Ignoring Sukias *et al.* (2001) TAN content (due to its unusually low total N value), there appears to be a clear trend of increasing TAN content (Table 2-6). This is due to increasing NH_4^+-N concentration of FDE. The concentration of NH_4^+-N in FDE is increasing at a faster rate than Total N. Higher TAN contents of an FDE, when comparing two FDE's with the same total N, are likely to promote higher leaching and N_2O losses when applied to soil due to a greater portion of N being available for plant uptake, leaching and denitrification. Both TAN content and NH_4^+-N concentration, and their trends, are important, however, the "raw" values of NH_4^+-N concentration are of greater interest as they directly relate the portion of NH_4^+-N to the volume of FDE, not as a portion of total N, which may vary. For example, providing application rate remains constant, an increase in NH_4^+-N concentration implies a greater NH_4^+-N loading, however, if the TAN content increases, this only indicates that the portion of NH_4^+-N is higher, relative to total N, and does not explain if NH_4^+-N concentration is increasing or total N concentration is decreasing.

Table 2-7 DM%, pH & Total C concentrations of FDE previously used in field trials.

Study	DM%	pH	Total C (g L^{-1})
Silva *et al.* (1999)		7.8	3.1
Degens *et al.* (2000)		6.3	6.3
Longhurst *et al.* (2000) (Review)	0.9		
Barton and Schipper (2001)	0.4	7.9	1.5
Di and Cameron (2002a)	0.9	7.6	2.2
Hawke and Summers (2003)		6.8	2.1
Bhandral *et al.* (2007) from 2003	0.1	7.9	3.2
Luo *et al.* (2008b)			0.9
Bhandral *et al.* (2010)		7.7	0.9
Houlbrooke *et al.* (2011)	1.2		1.3
Mean	0.7	7.4	2.4
Range	0.9-1.2	6.3-7.8	0.9-6.3

Means of DM%, pH and total C concentration of FDE from the literature are shown in Table 2-7 Other than concluding that the average pH of FDE is approx. 7.4, little accurate information can be obtained from Table 2-7 due to lack of valid data on these characteristics. A lot of FDE for research is now prepared by mixing dung, urine and water; however, this is unlikely to be representative of natural FDE.

Table 2-8 Nutrient concentrations of FDE previously used in field trials.

Study	Nutrient (mg L^{-1})					
	P	K	S	Ca	Mg	Na
Degens *et al.* (2000)	93	183				382
Longhurst *et al.* (2000) (Review)	69	370	65	177	39	54
Di and Cameron (2002a)	56					
Hawke and Summers (2003)	31	53		33	15	19
Bhandral *et al.* (2007) from 2003	28	178				
Houlbrooke *et al.* (2011)	145	500				
Mean	70	257	65	105	27	152
Range	31-145	53-500		33-177	15-39	19-382

Other than N, P and K are the most important nutrients in FDE, P is required by most soils for optimum plant growth, while K can accumulate in the soil and pasture and cause metabolic problems at calving and in early lactation (Longhurst *et al.*, 2000; Otago Regional Council, 2004). Therefore, P and K concentration have been well analysed, however, limited data is available on other nutrients contained in FDE (Table 2-8).

2.5.4 N_2O Emissions from FDE

Van der Weerden *et al.* (2016a) noted a lack of data on the N_2O EF's (N_2O emissions as a percentage of N applied) for FDE in New Zealand, as a relatively minor number of New Zealand studies have

analysed the influence of FDE on N_2O emissions. Li *et al.* (2015a) highlighted that there are no N_2O emission studies where one factor in the FDE is altered and all other factors kept constant. E.g. increasing C content has shown some relationship with the EF's but N usually increases as well (vice versa), therefore, it is hard to determine a change in the EF to a single factor. In addition, existing studies on FDE are limited in their scope of region, season and soil type. The N_2O EF's of the few existing FDE trials are highly variable, ranging from 0.01 to 4.93% of total N applied (Table 2-9). Soil N_2O emissions are a function of many variables particularly, N and C content, O_2 concentration (as indicated by WFPS or gas diffusivity), soil pH, temperature and microbial history (see section 2.3.4). The wide range of variables in these trials make them difficult to compare. No clear trends are drawn from the associated EF's (Table 2-9).

Table 2-9 Summary of New Zealand studies that measured N_2O emissions as influenced by FDE.

Study	Month applied	Soil moisture	N applied		N_2O emission factor	
		Mean (range) (% WFPS)	Total N (kg ha^{-1})	NH_4^+-N (kg ha^{-1})	Total N (% N applied)	NH_4^+-N
Barton and Schipper (2001)	Spring	76 (63-90)	50	18	1.20	3.33
	Autumn	50 (35-66)	50	18	0.15	0.42
	Spring	91 (79-100)	50	18	0.40	1.11
	Autumn	59 (44-85)	50	18	0.25	0.69
Bhandral *et al.* (2007)	Autumn	50 (35-58)	61	43	0.42	0.42
	Winter	65 (47-86)	49	29	0.16	0.28
Luo *et al.* (2008b)	Autumn	40 (30-53)	50	-	0.03	-
	Summer	30 (26-38)	50	-	0.01	-
Bhandral *et al.* (2010)	Spring	80 (61-90)	25	-	1.97	-
	Summer	80 (69-94)	21	-	4.93	-
Li *et al.* (2014)	Wint/spr	80 (70-100)	100	38	0.13	0.34
Li *et al.* (2015b)	Spring	70 (30-90)	98	69	1.65	2.35
	Summer	37 (20-65)	101	39	0.01	0.03
	Autumn	58 (55-65)	101	38	0.56	1.50
Li *et al.* (2016)	Winter	60 (20-70)	100	56	0.62	1.11
van der Weerden *et al.* (2016b)						
Experiment 1: 2013	Spring					
Waikato		72 (20-80)	58	31	0.11	0.21
Manawatu		68 (20-75)	52	15	0.78	2.70
Canterbury		52 (20-70)	56	37	0.06	0.09
Otago		77 (30-70)	57	31	0.14	0.26
Experiment 2: 2014	Spring					
Waikato		80 (40-100)	54	23	0.04	0.09
Manawatu		65 (15-75)	28	17	0.94	1.55
Canterbury		61 (50-80)	43	21	0.12	0.25
Otago		95 (40-100)	46	29	0.75	1.19

In contrast, it is clear that the NH_4^+-N concentration of FDE is highly variable, and with recent intensification of dairy farming, appears to be increasing (Table 2-6). The large number of variables involved in the application depth of FDE further enhance the variability of the rate of NH_4^+-N applied to pasture. However, despite this, no research has been conducted to analyse the influence of FDE NH_4^+-N concentration on soil N_2O emissions. Chadwick 2011 suggested that FDE NH_4^+-N concentration drives soil N_2O emissions, as against total N concentration of the FDE.

When analysing the data contained in Table 2-9, the EF does not appear to be influenced by either the rate of either total N or NH_4^+-N applied. Not only have no New Zealand FDE studies analysed the effect of increasing NH_4^+-N concentration on N_2O emissions, but none have analysed the influence of total N rate on N_2O emissions either.

The New Zealand study closest to examining the effect of N rate on N_2O emissions was Li *et al.* (2015b). They compared the influence of typical fresh FDE with FDE that had been stored for four months. Storing FDE is becoming more common now as farmers have to defer FDE irrigation (Dairy NZ, 2015). In spring, application of fresh FDE at 100 kg N ha^{-1} (70 kg NH_4^+-N ha^{-1}) produced a significantly higher EF of 1.65% of total N applied, compared with application of stored FDE at 60 kg N ha^{-1} (36 kg NH_4^+-N ha^{-1}), which produced an EF of 0.80%. This suggests that N_2O emissions increase exponentially with increasing N rates, however, other factors such as C may have also varied, and it is unclear whether or not the hydraulic loading (and hence WFPS) was equal for the two treatments. It was also not mentioned how the FDE was stored, therefore, it is not possible to determine any potential differences in bacterial populations that there may have been. In addition, the opposite occurred in summer, the stored FDE produced a significantly higher EF of 0.25% of the total N applied compared to the fresh FDE, which had an EF of 0.01%. In the same study, Li *et al.* (2015b) suggested that greater N_2O emissions from FDE compared to a synthetic fertiliser treatment could be attributed to higher inorganic-N content, however, this could not be distinguished from any WFPS effect.

Normally, studies involving FDE either compare the N_2O emissions from the FDE to N_2O emissions of other N inputs (e.g. Li *et al.* (2015b)) or compare them to amended FDE e.g. FDE with a nitrification inhibitor (e.g. Li *et al.* (2014)).

A review of the first four New Zealand studies by Laubach *et al.* (2015), where FDE was applied at rates between 21 and 100 kg N ha^{-1}, suggested that the EF would not be influenced by total N applied. In response van der Weerden *et al.* (2016b) predicted that the EF would likely remain unaffected up to 150 kg N ha^{-1}.

In contrast, when combining a variety of data, Li *et al.* (2015a) showed that the N_2O EF's increased exponentially in response to increasing rates of total N (Figure 2-8). However, this exponential conclusion needs to be interpreted carefully, as the data included some overseas studies, which may not be representative of New Zealand's farming systems. Also, a visual analysis of the graph shows large variation in the data and suggests the exponential curve is a relatively poor fit (R^2 = 0.164). A linear relationship also appears possible, if not a better fit than the exponential curve.

In addition, often an increase in total N is also associated with an increase in C and DM%, so not only is it is difficult to partition the effect of a single variable, but this is less relevant to the concentration of NH_4^+-N, as NH_4^+-N concentration is more independent of these variables (Table 2-6, 2-7). Therefore, the conclusion drawn by Li *et al.* (2015a), may not accurately represent the effect of solely increasing total N or NH_4^+-N rates. Never the less, Figure 2-8 shows that, in this data-set, the EF increases with N application rate and therefore, N_2O emissions have increased exponentially in response to N application rate.

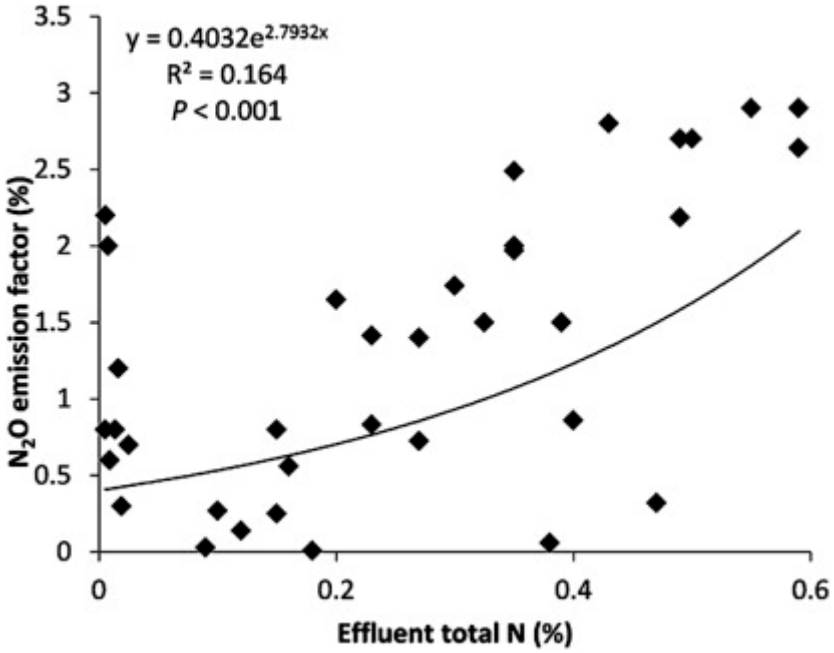

Figure 2-8 Relationship between total N content of FDE applied and soil N_2O emissions from Li *et al.* (2015a).

As the N fraction of FDE and other N inputs has historically been measured by total N, little information on the influence of NH_4^+-N concentration on soil N_2O emissions is available, irrespective of the type of N input. Urine is largely urea [$CO(NH_2)_2$], which rapidly hydrolyses to NH_4^+ in the soil (Haynes *et al.*, 1986). There is more information on the EF from urine, however, the rates of urine applied in a urine patch, up to 500 kg N ha^{-1} for sheep and 1000 kg N ha^{-1} for dairy cows, are far in

excess of those that FDE is regulated to (Di and Cameron, 2002b; Environment Canterbury Regional Council, 2015).

In response to the lack of data on the influence of the N_2O EF's of NH_4^+-N rate, total N is used as a proxy for predicting the influence of FDE NH_4^+-N rate on soil N_2O emissions. However, Shcherbak *et al.* (2014) noted that many studies, irrespective of the type of N input, only use 2-3 rates of N, which are insufficient to accurately determine any relationship between the N applied and N_2O emissions.

As already established no trials have analysed the effect of FDE total N rate on N_2O emissions in New Zealand. Surprisingly, this is also true for the rest world. No evidence of any FDE N application rate trial, where N_2O emissions are measured, can be found globally.

Although there are differences between dairy and pig effluent, Rochette *et al.* (2000) noticed the EF increased from 1.23 to 1.65% when pig slurry application rate doubled. However, Mkhabela *et al.* (2009) observed no increase in the EF when N application rate of pig slurry increased. A third pig slurry study by Velthof *et al.* (2003) had previously reported results in agreement with Mkhabela *et al.* (2009), N_2O emissions increased linearly with N application rate and no increase in the EF was observed.

A metaanlysis of 78 N application rate studies found that the N_2O response to N inputs increased exponentially with increasing N_2O emissions as N inputs increased above crop requirements (Shcherbak *et al.*, 2014). The authors suggested that small additions of N to low input systems will have little impact on N_2O emissions, however, similar additions in intensively fertilised systems will have a far greater impact on emissions, highlighting the different points of the response curve that each system is located. A non-linear relationship takes into account the biological thresholds that may occur due to plant-bacteria competition e.g. when the availability of soil inorganic-N exceeds crop N demands there will be surplus N for bacteria to use and produce N_2O. The percentage change in the EF was significantly lower for controlled release urea (CRU) than for other synthetic fertilisers and lower (although not significantly) for split vs single fertiliser applications. Indicating there is less surplus N for N_2O emission from slow release N inputs (Shcherbak *et al.*, 2014).

This is in agreement with van Groenigen *et al.* (2010) who conducted a metaanalysis of N application rate studies of non-leguminous crops. The N_2O produced was normally linearly proportional to N application rate when N was applied within crop requirements (approximately 180 – 190 kg N ha^{-1}), however, yield-scaled N_2O emissions increased more than threefold at a surplus of 90 kg N ha^{-1}. Hoben *et al.* (2011) observed a non-linear response of N_2O to N fertiliser application rate on average, although a linear response was observed at some sites. Emission factors increased significantly

above the local recommended application rate of 135 kg N ha^{-1}, but this is likely to be crop specific. An N fertiliser application rate laboratory study provided further support for a non-linear relationship with N_2O emissions, N_2O as a percentage of total inorganic-N increased from 0.1 to 0.7% when N increased from 50 to 150 kg N ha^{-1}.

It appears that the EF does increase as the rate of N applied increases, suggesting that NUE decreases with increasing N rate due to N exceeding plants requirements and becoming relatively more available for loss as N_2O by nitrification or denitrification. FDE is a slow release form of N due to the organic N from the dung component, therefore, N in excess of plants requirements and conducive to N_2O production will be minimal. Hence increases in N application rate of FDE are expected to produce no, or reduced increases in the EF's (if any), similar to the CRU treatment in the study by Shcherbak *et al.* (2014). Therefore, despite increasing evidence that N_2O emissions increase exponentially with increases in the rate of N applied (particularly N fertiliser), it is expected that the response of N_2O emissions to FDE application rate, within regulatory constraints, will be linear. At rates above 200 kg N ha^{-1} N_2O emissions from FDE may be exponential, however, at the rates typically applied and regulated to in New Zealand (see section 2.5.2), responses are likely to be in the near linear phase of any such exponential relationship, based on the thresholds outlined above.

2.6 Conclusion of Literature Review

In conclusion, N_2O emissions have an adverse effect on the atmosphere. They contribute to climate change and destruction of the O_3 layer. FDE is thought to be a major source of N_2O emissions in New Zealand, however, little research has been conducted to understand the processes and magnitude of such losses.

Application of FDE to land is regulated to minimise NO_3^--N leaching, by restricting application depth and total N concentration. No consideration is given to the influence of FDE on gaseous emissions such as N_2O. The literature is clear that characteristics of FDE, especially the NH_4^+-N concentration are highly variable. It has been suggested that FDE NH_4^+-N concentration is a key driver of N_2O emissions, however, no research has been conducted to confirm this. Understanding how variables of FDE influence soil N_2O emissions is critical to developing strategies to mitigate such losses. In addition, information about the N_2O EF's from FDE is necessary to accurately calculate New Zealand's greenhouse gas inventory.

This research will analyse the effects of solely varying the NH_4^+-N content of FDE on soil N_2O emissions. It is expected that N_2O emissions will increase linearly with NH_4^+-N content, indicating the available N component of FDE is driving N_2O emissions.

Chapter 3

Materials and Methods

3.1 Experimental site

The experiment site was a perennial ryegrass (*Lolium perenne* L.)/ white clover (*Trifolium repens* L.) pasture growing on a Wakanui silt loam (Mottled Immature Pallic Soil) (Landcare Research, 2016) in paddock 12 of Iversen Field at Lincoln University, Canterbury, New Zealand (43.64836° S, 172.46794° E, 11 m.a.s.l.) . Physical and chemical characteristics of the soil can be found in Table 3-1. The soil is imperfectly drained and has a category B dairy effluent (FDE) risk rating (Table 2-5) (Landcare Research, 2016).

Table 3-1 Basic soil properties (0-7.5cm depth)

Soil properties	Value
pH	6.1
Olsen P (mg/L)	18
Organic matter (%)	5.2
Total carbon (%)	3
Total nitrogen (%)	0.28
C:N ratio	10.6
Anaerobically mineralisable N (µg/g)	91
Anaerobically mineralisable N to total nitrogen ratio (%)	3.2
Sulphate sulphur (mg/kg)	3
Potassium (me/100g)	1.07
Calcium (me/100g)	7.1
Magnesium (me/100g)	1.36
Sodium (me/100g)	0.24
CEC (me/100g)	14
Base saturation (%)	72
Bulk density (g/cm^3)	1.24

Normally the site was used for 'cut and carry' and the pasture had not been grazed with stock since the 14th April 2015, 1 year prior to the trial commencing. Fencing continued to exclude stock from the site during the trial. This prevented inputs of N from animal's, dung and urine, influencing the results.

3.2 Site preparation and treatments

Six treatments were replicated four times in a completely randomized block design (Figure 3-1 and Figure 3-2).

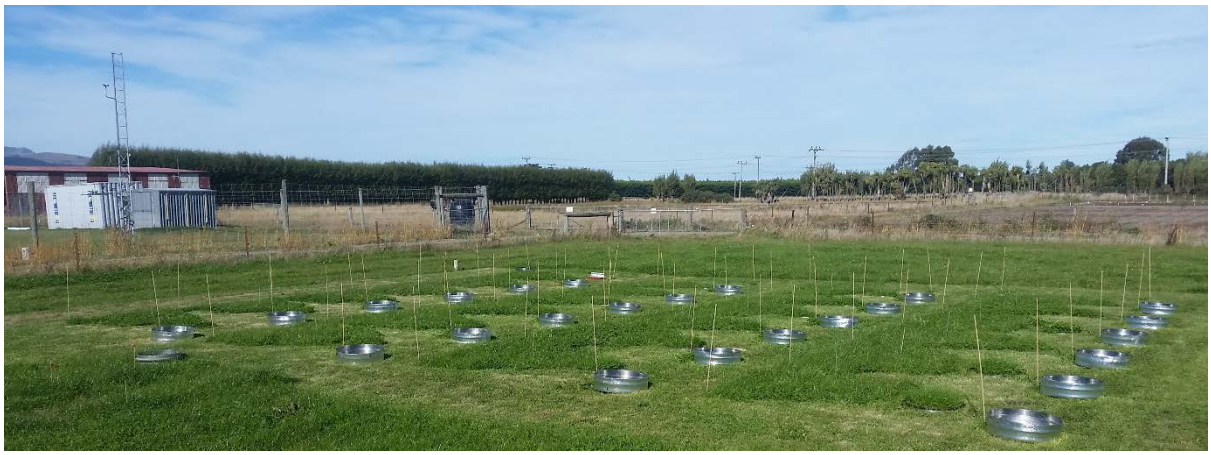

Figure 3-1 Looking SE towards the experiment (on day 12 of the trial) in paddock 12 of Iversen Field at Lincoln University.

Details of site preparation for the trial are described in Table 3-2.

Table 3-2 Site preparation

Event	Day
Existing herbage mown with a tractor and removed from the site	-22
60 mm of irrigation applied to begin softening the soil for chamber installation	-18
Site mown with a ride-on lawnmower to a height of 5cm, herbage was removed and represented a grazing event.	-8
30 mm irrigation applied to soften the soil for chamber installation	-7
Chambers installed later that day	-7
Treatments applied	0

The trial was marked out into four blocks, each with six plots, 24 plots in total (Figure 3-2). A stainless steel gas chamber (370 mm in diameter) was inserted 10cm into the ground in one half of each plot. Whilst an area the same size as the chamber was marked out with wooden pegs for soil sampling in the second half of the plot (Figure 3-3).

	Block 1		Block 2		Block 3		Block 4
6	Control	*7*	200	*18*	200	*19*	90
5	300	*8*	300	*17*	300	*20*	150
4	200	*9*	400	*16*	90	*21*	200
3	90	*10*	150	*15*	150	*22*	400
2	400	*11*	90	*14*	400	*23*	300
1	150	*12*	Control	*13*	Control	*24*	Control

N ↑

5 = Plot number

300 = Treatment

Figure 3-2 Block and Treatment layout of the trial.

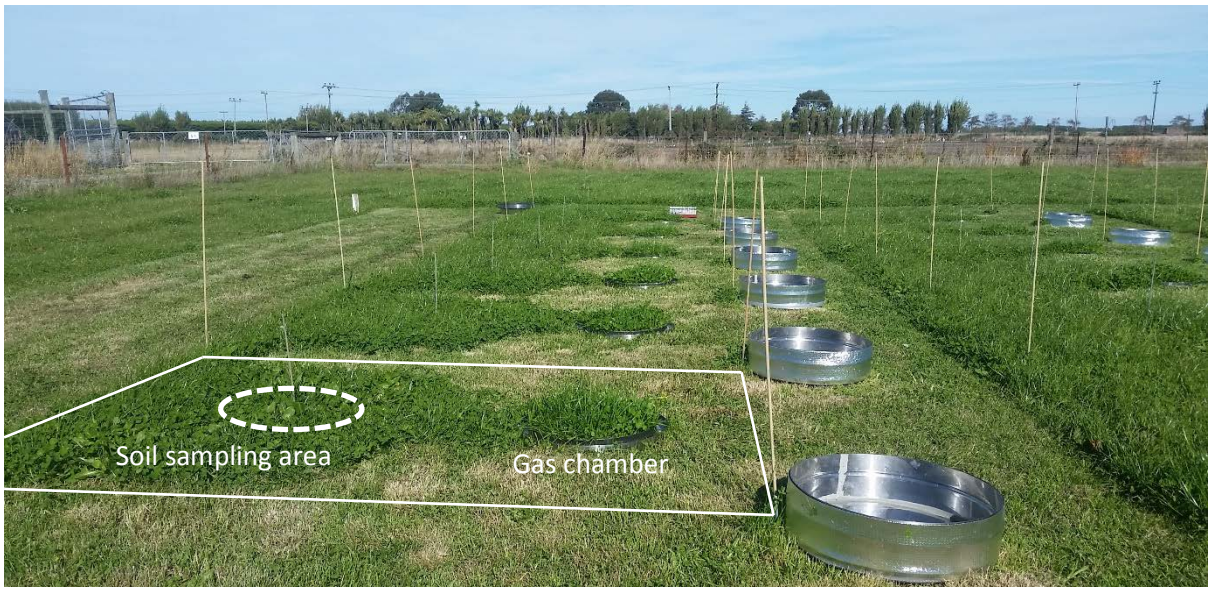

Figure 3-3 Soil sampling areas (marked with a short peg) and gas chambers of plots 19 (foreground) to 24 (background). Chamber lids are sitting inverted to the right of each plot.

Treatments consisted of a control or farm dairy effluents (FDE) at containing NH_4^+-N at different rates: 90, 150, 200, 300 or 400 mg NH_4^+-N L^{-1}. Exact concentrations of NH_4^+-N applied in the FDE treatments can be found in Table 3-3. FDE was applied at an application depth of 10 mm based on soil properties, application method and industry best practice to minimise NO_3^--N leaching and surface runoff (Houlbrooke *et al.*, 2013; Landcare Research, 2016). The control had 10 mm of water applied to keep WFPS constant.

FDE was collected from the sump on the South block of the Lincoln University dairy farm on day -2. FDE in the sump was mixed prior to collection by pumping and returning the FDE to the sump for 1-2 minutes to ensure that the FDE collected was representative of that applied to pasture. An excess of c.a. 200 L of FDE was then pumped from the sump into a 300 L fully enclosed tank. FDE was sub-sampled immediately and the remainder stored in the shade for two days. Sub sampled FDE was centrifuged at 2000 rpm for 10 minutes then syringe filtered through 0.45 µm filters (CA+GF, Phenex), then analysed for NH_4^+-N using flow injection analysis (FOSS FIAstar 5000 twin channel analyser, Foss Tecator AB). Analysis of the FDE returned an average NH_4^+-N value of 87 (90) mg NH_4^+-N L^{-1}, upon which treatment rates were based from.

Ammonium sulphate solution was prepared at rates of 60, 110, 210 and 310 mg N L^{-1} to raise the base FDE NH_4^+-N concentration to rates of c.a. 150, 200, 300 and 400 mg NH_4^+-N L^{-1}. Ammonium sulphate solution was mixed with FDE immediately prior to treatment application. The required mass of anhydrous ammonium sulphate was added to 100 ml volumetric flasks and brought up to 100 ml with de-ionised water then gently shaken. Ten atom% ^{15}N ammonium sulphate was used for

the 150 and 400 mg NH_4^+-N treatments in order to determine the FDE's contribution to N_2O emissions.

3.3 Treatment application

A total of 1.075 L of liquid (water, FDE or $(NH_4)_2SO_4$ amended FDE) was applied to each 0.1075 m^2 chamber or soil sampling area to give the application depth of 10 mm. The liquid was applied by slowly pouring from a 3 L measuring jug in a spiral pattern. When applying treatments to the soil sampling areas a spare chamber ring was pressed firmly onto the perimeter of the soil sampling area, then removed only once the liquid had infiltrated into the soil, to prevent seepage of the treatment outside of the soil sampling area. Treatments were applied in the order of control, 90, 200, 300, 150 and 400 mg NH_4^+-N L^{-1} to prevent contamination with; FDE, higher rates of N and ^{15}N.

- For the control treatments 1.075 L water was applied to keep the WFPS constant with FDE treatments.

- For FDE treatments; FDE collected 2 days prior was thoroughly mixed with a garden spade then c.a. 5 L for each treatment, for each sampling area, was removed with a 5 L measuring jug. Then 1.075 L of FDE from the 5 L measuring jug was poured into a 3 L measuring jug, mixing well with a plastic rod before each delivery.

    - In the case of the 90 mg NH_4^+-N treatments the 1.075 L of the base FDE was applied, unamended, to the chamber;

    - while in the case of $(NH_4)_2SO_4$ amended FDE treatments 20 ml of the required ammonium solution was added to the 1.075 L of base FDE using a 30 ml syringe, then gently stirred and applied to the desired chamber.

- For each 5 L of effluent this process was repeated for each of the 4 replicates of the treatment concerned, then 0.54 L of the remaining FDE was mixed with 10 ml of the remaining ammonium solution (creating an equal dilution to treatments applied), with a 400 ml sample collected for analysis.

- Any remaining FDE in the 5 or 3 L jugs was discarded, then another 5 L of FDE was removed from the tank and the process repeated with the same rate of ammonium solution for the soil sampling area or a different rate of ammonium solution for the subsequent treatment.

3.4 Effluent Analysis

Two 400 ml samples of each treatment were collected for analysis. One of the samples for each treatment was sub sampled and prepared for NH_4^+-N analysis as above (which was frozen and analysed later), additional sub samples of ^{15}N treatments were prepared in the same way for ^{15}N analysis. The second sample was frozen directly, then brought to room temperature for sub-sampling and analysis four months later. Each sample was shaken well, its pH measured using a pH meter with electrode (SevenEasy pH meter with and InLab Expert Pro electrode, Mettler Toledo), then poured into a round flat-bottomed 1 L vessel and stirred with a magnetic stirrer. FDE was subsampled using an eye dropper (narrow end removed) and placed in either a tared aluminium tin for determination of the DM content or 30 ml vials for chemical analysis.

DM% was calculated by dividing the mass of FDE remaining after drying in an oven at 100°C for 48 hrs by its wet mass. Total C and N of the FDE were analysed using an Elementar Vario-Max CN Elemental Analyser (Elementar GmbH). P, K, S, Ca, Mg, Na, Al, Mn, Cu and Zn were determined by firstly digesting the FDE (CEM MARS Xpress, CEM Corporation), then analysing the product using an inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Varian 720 ICP-OES, Varian Australia PTY Ltd). A stable isotope ratio mass spectrometer (CFIRMS, Sercon) was used to confirm the ^{15}N content of the ^{15}N amended FDE. The 150 and 400 mg NH_4^+-N treatments had ^{15}N enrichments of 2.79 and 7.25 atom%, respectively.

Characteristics of both the base FDE and final treatments applied is described in Table 3-3.

Table 3-3 Characteristics of base FDE; nutrient concentrations of base FDE; Total N, NH_4^+-N and Total C applied, TAN content and C:N ratio of FDE treatments.

Characteristics of base FDE

Total solids (%)	pH	Total C (mg L^{-1})	Total N (mg L^{-1})	NH_4^+-N (mg L^{-1})	TAN[a] (% of Total N)	C:N ratio
0.31	7.75	1010	160	91.3	57.1	6.382

Nutrient concentration of base FDE (mg L^{-1})

P	K	S	Ca	Mg	Na	Al	Mn	Cu	Zn
32.3	183.7	24.9	124.0	34.8	53.0	11.5	0.9	0.2	0.6

Total N, NH_4^+-N and Total C applied, TAN content and C:N ratio

	Total N applied			NH_4^+-N applied			TAN[a] content	Total C	C:N
Treatment	(mg L^{-1})	(mg $plot^{-1}$)	(kg ha^{-1})	(mg L^{-1})	(mg $plot^{-1}$)	(kg ha^{-1})	(% total N)	(mg L^{-1})	ratio
90	162(13)ǂ	174	16	91(1)	98	9	56	1008	6.2
150	233(5)	250	23	150(15)	161	15	64	973	4.2
200	247(14)	266	25	207(16)	222	21	84	766	3.1
300	337(1)	363	34	298(2)	320	30	88	965	2.9
400	397(5)	427	40	394(14)	424	39	99	1021	2.6

[a] TAN = total ammoniacal N.

ǂ Numbers in parentheses are the standard error of the means, (n = 3).

The analysed total N concentration of the higher rates of $(NH_4)_2SO_4$ amended FDE did not represent the theoretical total of 162 mg N L^{-1} in the base FDE plus the concentrations of NH_4^+-N added e.g. for

the 400 mg NH_4^+-N L^{-1} treatment 310 mg NH_4^+-N was added to the base FDE (162 mg N L^{-1}), indicating a theoretical Total N value of 472 mg N L^{-1}, significantly more than the 397 mg N L^{-1} value provided by analysis of the FDE. This could have been due to inaccuracies in methods of both the sub-sampling and the analysis, or the assumption of 1% N = 10g L^{-1} may not be valid. Never the less, analysed values (converted to mg L^{-1} as NH_4^+-N was reported in mg L^{-1} when analysed) were used for calculations.

NO_3^--N content of the FDE was negligible, < 1 mg L^{-1}. The use of ammonium sulphate to raise the NH_4^+-N level of FDE increased sulphur concentrations from 24.9 mg L^{-1} in the 90 mg NH_4^+-N treatment to 101.6, 173.0, 315.4 and 433.4 mg L^{-1} in the 150, 200, 300 and 400 mg NH_4^+-N treatments, respectively.

3.5 Soil sampling

Soil sampling occurred on days 1, 7, 14, 21, 28 and 35. On each sampling day a soil corer (7.5 cm depth by 2.5 cm diameter) was used to remove two adjacent soil cores from each soil sampling area. On subsequent soil sampling days' soil cores were taken a distance from previous cores to the avoid effects from the previous cores (Figure 3-4). The two soil cores were combined, stored at 4°C and processed within 48 hrs of sampling.

Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations were determined by extracting 4 g soil with 40 ml 2.667 M KCl. Samples were shaken end over end for 1 hr, centrifuged for 10 mins at 2000 rpm then gravity filtering through Watmann no. 42 filter paper.

Dissolved organic-C (DOC) was determined by extracting 10 g of soil with 100 ml de-ionised water. Samples were shaken side to side at 75 rpm for 30 mins, centrifuged at 3300 rpm for 30 mins and then syringe filtered through 0.45 µm filters (CA+GF, Phenex).

All soil extractions were frozen until analysis. NH_4^+-N and NO_3^--N extractions were analysed using flow injection analysis as per FDE above and DOC extractions were analysed by a total organic-C analyser (TOC-5000A, Shimadzu Oceania Pty ltd).

The following equations were used to calculate gravimetric water content (Θ_G), volumetric water content (Θ_V), total porosity (ϕ), WFPS, and gas diffusivity D_P/D_O, to understand water content and gas diffusion in the soil.

$$\theta_G = \frac{(mass\ wet\ soil - mass\ dry\ soil)}{mass\ dry\ soil} \quad \textbf{(13)}$$

$$\theta_V = \theta_G \times B_D \quad \textbf{(14)}$$

$$\phi = 1 - \left(\frac{B_D}{2.65}\right) \quad \textbf{(15)}$$

$$WFPS = \frac{\theta_V}{\phi} \quad \textbf{(16)}$$

$$D_P/D_O = 0.5\,\phi\left(\frac{\epsilon}{\phi}\right)^{2+1.38\,\phi} \quad \textbf{(17)}$$

Where B_D = bulk density. Dry soil had been oven dried at 105°C for 48 hrs.

Figure 3-4 Relative locations of the 6 pairs of soil cores removed throughout the 35-day trial period. The chamber ring was used in the photograph to indicate the area the FDE was applied to.

3.6 Nitrous Oxide (N_2O)

3.6.1 Flux measurement

Following treatment application N_2O fluxes were determined daily, for days 0-7 (except day 4 due to inclement weather) and then days 10, 12, 14, 16, 17, 19, 22, 23, 24, 26, 28, 31, 33 and 35. A 35-day gas flux sampling period was deemed sufficient for nitrification and denitrification to have ceased due to the low rates of N applied and flux results, that showed a lack of emissions from day 10 onwards, even when 20 mm of water was applied on day 21. Gas sampling commenced at 10:30 a.m and occurred until approximately 11:45 a.m, during which period gas fluxes (influenced by diurnal variation) are at an average for the day (Van Der Weerden *et al.*, 2013). At gas sampling events external annular moats on the chamber bases were filled with water to create a gas-tight seal when

the lid (11 cm deep by 42.5 cm diameter) was fitted. To begin the sampling-process the lid (with septum) was placed over the gas chamber creating a headspace of 0.0149 m^3. Gas samples were taken at t_0 (0 min), t_1 (25 min) and t_2 (50 min) after the lid was fitted. Samples were removed from the headspace using a 30 ml plastic syringe fitted with a 3-way stop-cock and long needle, that extended into the headspace, and immediately transferred (Figure 3-5) to previously evacuated (Figure 3-6). (-1 atm) 6 ml Exetainers (Labco Ltd.).

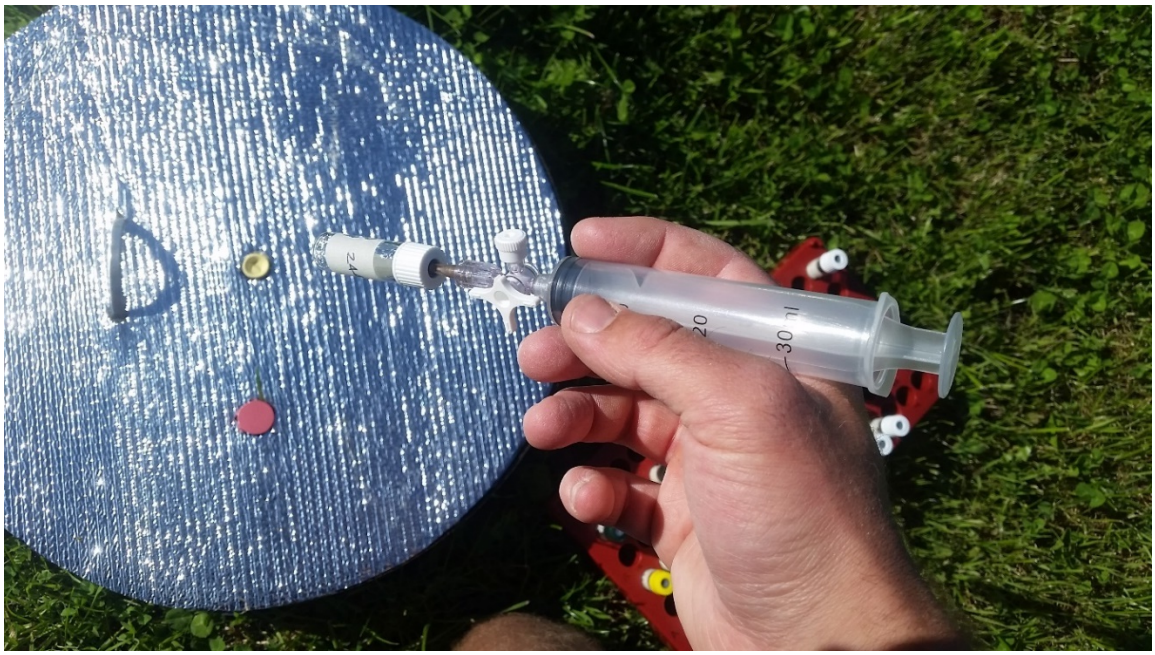

Figure 3-5 Transferring a gas sample to the previously evacuated 6 ml Exetainer.

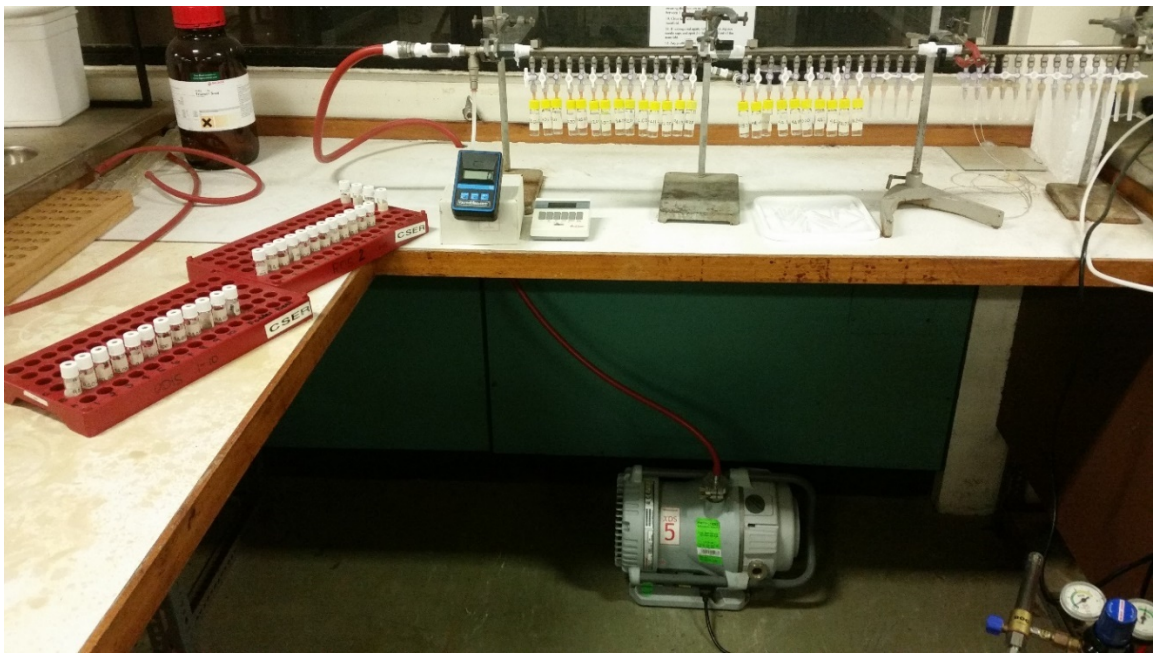

Figure 3-6 Evacuating Exetainers prior to gas sampling using a specially designed vacuum pump system in the laboratory.

Gas samples were analysed using a gas chromatograph (GC Model 8610C, SRI Instruments). Lids remained on the gas chambers until 1:30 p.m (3 hrs) at which time a single sample was taken from

the headspace to analyse for ^{15}N. $^{15}N_2O$-N gas sampling occurred by the same method as ordinary gas sampling, described above. However, $^{15}N_2O$-N gas samples were collected in 12 ml Exetainers flushed with Helium (He) prior to evacuation. Lids were removed from chambers following ^{15}N gas sampling (c.a. 2 pm). ^{15}N gas samples were analysed on the mass spectrometer as per the ^{15}N FDE samples above.

3.6.2 Flux calculations

Three samples taken at t_0, t_1 and t_2 following covering allowed calculation of the flux both for linear and non-linear increases of N_2O concentration during gas collection. The two conditions (linear and non-linear) are illustrated in Figure 3-7.

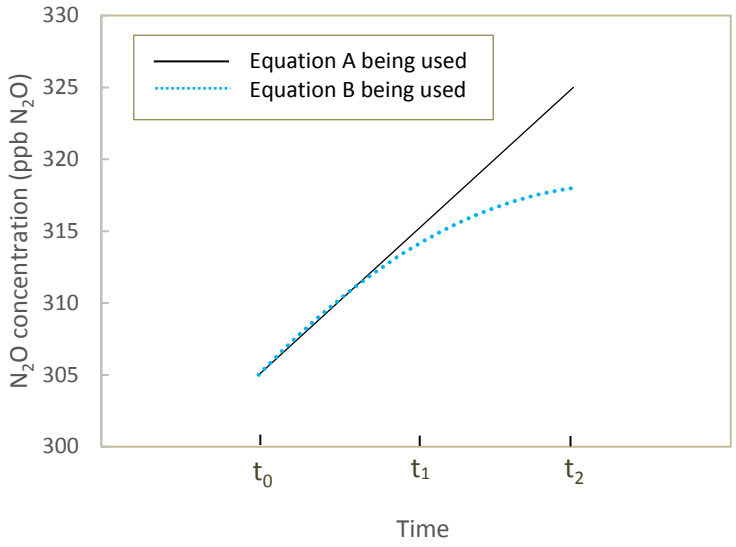

Figure 3-7 Typical nitrous oxide increases beneath the cover illustrating the use of either equation A or equation B.

The N_2O flux was calculated by:

$$F = \begin{cases} \frac{(C_1 - C_0)}{(C_2 - C_1)} \leq 1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqt.A \\ \frac{(C_1 - C_0)}{(C_2 - C_1)} > 1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqt.B \end{cases} \quad \textbf{(18)}$$

With,

$$F_{Ept.A} = \left\{ \frac{(C_2 - C_0)\, V_C \,\mathrm{P}\, C_{ha}\, C_D\, M_{N2}}{G_C\, (T_K + T^{\circ}C)\, A_C\, t_2} \right. \quad \textbf{(19)}$$

$$F_{Ept.B} = \frac{V_{C(C_1 - C_0)^2}}{(2C_1 - C_2 - C_0)} \ln\left[\frac{(C_1 - C_0)}{(C_2 - C_0)}\right] \frac{\mathrm{P}\, C_{ha}\, C_D\, M_{N2}}{G_C\, (T_K + T^{\circ}C)\, A_C\, t_1} \quad \textbf{(20)}$$

A description of constants and the associated values used to calculate N_2O fluxes in this experiment is provided in Table 3-4.

Table 3-4 Constants and the values used to calculate N_2O fluxes.

Constant	Description	Unit	Value
	Chamber (Lid) radius	m	0.2125
	Chamber (lid) height	m	0.1053
	Ring radius	m	0.1850
F	N_2O flux	g N_2O-N ha^{-1} day^{-1}	
P	Atmospheric pressure	Pa	102274
V_C	Chamber Volume	m^3	0.0149
A_C	Ring Area	m^2	0.1075
G_C	Gas Constant	J K^{-1} mol^{-1}	8.314
T_K	Absolute Temp	K	273.15
$T_{°C}$	Air Temp	°C	
C_{ha}	Conversion m^2 to ha		10000
C_D	Minutes per day	min	1440
M_{N2}	Molecular weight N_2O-N	g mol^{-1}	28.0134
t_0	Start of cover period	min	0
t_1	Half of cover period	min	25
t_2	Total cover period	min	50
C_0	N_2O flux t_0	ppm_v	
C_1	N_2O flux t_1	ppm_v	
C_2	N_2O flux t_2	ppm_v	

Cumulative fluxes (CUM. F.) for the 35-day trial period were calculated through integrating daily fluxes by trapezoidal interpolation. Whereby the equation:

$$CUM.F.(g\ N_2O - N\ ha^{-1}) = \sum[(X_2 - X_1)Y_1 + \frac{(X_2 - X_1)}{2}(Y_2 - Y_1)] \quad \textbf{(21)}$$

Was used to calculate the area of each component under the curve, and then sum the areas of each component to derive the cumulative flux of the treatment.

Emission factors (EF) for each treatment (T) were calculated using the equation:

$$EF_T = \frac{(Cum.F._T - Cum.F._C)}{Total\ N\ or\ NH_4^+ - N\ applied} \quad \textbf{(22)}$$

Where the cumulative flux of the treatment was subtracted from the cumulative flux of the control (C) then divided by either the Total N or NH_4^+-N applied.

^{15}N recovery was calculated using the equation:

$$\%\,^{15}N\ recovery = \frac{(moles\ N\ in\ sample \times (atom\%\,^{15}N\ sample - atom\%\,^{15}N\ control))}{(moles\ N\ applied \times (atom\%\,^{15}N\ applied - atom\%\,^{15}N\ control))} \times 100 \quad \textbf{(23)}$$

3.7 Pasture

Non sampling areas of the trial were mown as required. Chambers were harvested using hand shears, cutting to a height of 5 cm on day 19. Figure 3-8 A and B, illustrates the pasture before and

after it was harvested. All pasture removed from the chamber was collected and its fresh weight recorded. Dry weight of the sample was recorded following drying in a forced draught oven at 60°C for 48 hrs. Dry matter content of each sample was calculated by dividing its dry weight by its fresh weight. Soil sampling areas were mown to a height of 5 cm with a lawn mower on the same day to ensure they experienced the same conditions as the chambers. Care was taken to ensure the wheels of the lawn mower did not pass over the sampling areas and cause compaction of the soil.

Figure 3-8 Chamber 1 before and after harvesting on day 19.

Chambers were harvested again on the final day of the trial (day 35) using the same method as Cut 1, but cutting pasture as low as possible, to 1-2 cm, to obtain the lower component of the pasture for analysis (Figure 3-9 A and B). Fresh and dry weights and DM% of the herbage were also recorded as per Cut 1.

Figure 3-9 Harvesting chamber 1 on day 35. A; Using the hand shears to cut and collect the pasture, B; chamber 1 harvested as low as possible (1-2 cm high).

Oven dry (60°C) pasture samples from blocks 1, 3 and 4 from Cut 1 were ground then analysed for N and ^{15}N atom% on a mass spectrometer.

Nitrogen taken up per plot (g) was calculated by multiplying the dry mass of pasture harvested from the plot in Cut 1 by its N content (%N).

Apparent N and NH_4^+-N uptake was calculated by the equation:

$$Apparent\ N\ or\ NH_4^+{}_T = \frac{(N\ plot._T - N\ plot._C)}{Total\ N\ or\ NH_4^+ - N\ applied} \quad \textbf{(24)}$$

Where the N uptake per plot (N plot) of treatment (T) concerned was subtracted from the N uptake per plot of the control and divided by either the rate of N or NH_4^+-N applied to the plot.

^{15}N recovery of the pasture in Cut 1 was also calculated using equation 23 as used for the recovery of ^{15}N in the N_2O.

3.8 Irrigation

Due to lack of rainfall a hand shift irrigator with raised pivoting nozzles was used to simulate 20 mm of rainfall on day 21, in an attempt to trigger denitrification in the soil (Figure 3-10). Soil sampling, where by gravimetric water content and WFPS were calculated from samples, occurred prior to irrigation on day 21.

Figure 3-10 Irrigating the trial with 20 mm water on day 21 using a hand-shift irrigator with raised pivoting nozzles.

3.9 Statistical analysis

Statistical analyses were performed using the analysis of variance (ANOVA) directive of GenStat® (16th edition). The least significant difference (LSD) was used to determine statistical variation between the sample means at the 5% significance level. The standard error of the mean (SEM) was calculated by dividing the standard deviation of the sample by the square root of the number of samples. Where lower variation from the control treatment appeared to violate the assumption of normality of the data, the control was separated as a factor and analysed accordingly.

Chapter 4

Results

4.1 Climate data

Rainfall following treatment application was minimal, with a total of 14.4 mm over the 35-day trial period. Air temperature averaged 13.0°C (1.4-23.2) and soil temperature averaged 13.4°C (11.3-15.4) (range in brackets). Rainfall was on average 42.8 mm lower and air temperature on average 1.4°C higher than the weighted average of the 10-year means for April and May (National Institute of Water and Atmospheric Research, 2016). Due to low rainfall, plots were irrigated with 20 mm on day 21.

As desired, no significant differences in the WFPS were observed between treatments on days 1, 7, 14, 28 and 35 (Figure 4-1). The 150 mg NH_4^+-N L^{-1} treatment had a mean WFPS significantly lower ($P = 0.008$) than the 0, 90 and 200 mg NH_4^+-N L^{-1} treatments on day 21. WFPS was on average 68% for all plots 14 hrs after 10 mm of liquid was applied at treatment application, decreasing to on average of 58% for all plots by day 7. By day 21 average WFPS for all plots was 44%, 20 mm of irrigation was applied later that day, post WFPS measurement, in an attempt to trigger denitrification. Average WFPS for all plots rose to 53%, following irrigation, although this measurement was taken 7 days after the irrigation.

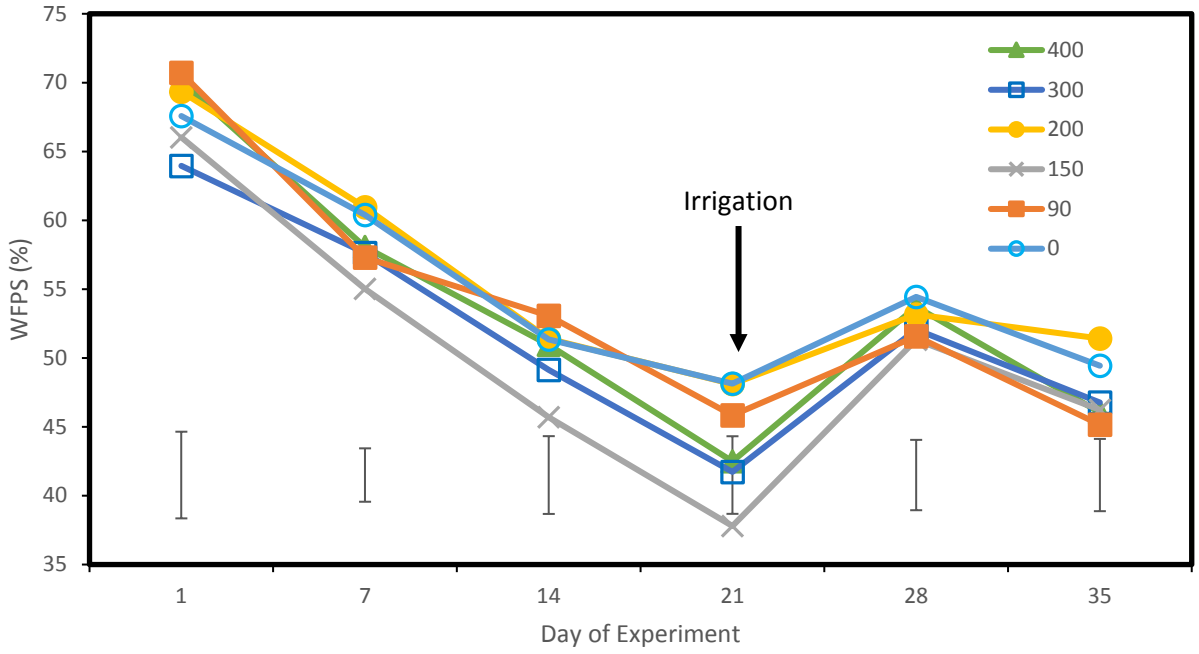

Figure 4-1 Mean (n = 4) water-filled pore space (WFPS) 0-7.5 cm depth, over time for the control (0) and FDE treatments (90, 150, 200, 300 and 400 mg NH_4^+-N L^{-1}). Vertical bars are the LSD (P=0.05).

Similar to WFPS, no significant differences in modelled relative gas diffusivity were observed between treatments on days 1, 7, 14, 28 and 35 (Table 4-1). The 150 mg NH_4^+-N L^{-1} treatment had a mean gas diffusivity significantly higher (P = 0.003) than the 0, 90 and 200 mg NH_4^+-N L^{-1} treatments on day 21. Gas diffusivity was on average 0.012 for all plots 14 hrs after 10 mm of liquid was applied at treatment application, increasing to an average of 0.025 for all plots by day 7. Addition of 20 mm of irrigation, post gas diffusivity measurement, on day 21 decreased the average gas diffusivity of all plots from 0.056 on day 21 to 0.035 on day 28, although again the measurement on day 28 was seven days after irrigation was applied.

Table 4-1 Mean (n = 4) modelled relative gas diffusivity (D_P/D_O) 0-7.5 cm depth, over time for the control (0) and FDE treatments (90, 150, 200, 300 and 400 mg NH_4^+-N L^{-1}).

Treatment	Gas diffusivity					
(mg NH_4^+-N L^{-1})	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
0	0.013	0.022	0.039	0.046	0.032	0.042
90	0.009	0.026	0.034	0.050	0.037	0.053
150	0.014	0.030	0.051	0.073	0.038	0.049
200	0.011	0.020	0.038	0.045	0.034	0.037
300	0.016	0.025	0.042	0.061	0.036	0.048
400	0.011	0.025	0.039	0.059	0.033	0.050
	NS	NS	NS	*	NS	NS
LSD (0.05)ɬ	0.005	0.007	0.013	0.013	0.011	0.013

* $P < 0.05$.

ɬ The least significant difference (LSD) between treatment means is shown for a 5% level of significance, *P* values > 0.05 = nonsignificant (NS).

4.2 Soil inorganic-N and dissolved organic-C

Maximum mean soil NH_4^+-N concentrations (0-7.5 cm) for all treatments (except 90 mg NH_4^+-N L^{-1}) occurred on day 1 (Figure 4-2 A). Soil NH_4^+-N concentrations had decreased by day 7, ranging between 1.3 and 3.2 mg kg dry $soil^{-1}$ for the remainder of the trial. Mean soil NH_4^+-N concentrations in the 400 mg NH_4^+-N L^{-1} treatment were significantly higher (P<0.001) than all other treatments on day 1 (15.2 mg kg dry $soil^{-1}$), however, no other treatments were significantly higher than the control.

Maximum soil NO_3^--N concentrations (0-7.5 cm) also occurred on day 1 (Figure 4-2 B). The FDE NH_4^+-N rate affected (P<0.05) soil NO_3^--N concentrations on days 1 and 7, with higher values in the 300 and 400 mg NH_4^+-N L^{-1} treatments of 12.6 and 9.7 mg kg dry $soil^{-1}$, respectively, on day 1 (P = 0.012), and 4.8 and 3.9 mg kg dry $soil^{-1}$, respectively, on day 7 (P = 0.024). Soil NH_4^+-N concentrations, particularly of the 300 and 400 mg NH_4^+-N L^{-1} treatments, continued to decrease to a range of 1.3 to 2.3 mg kg dry $soil^{-1}$ up until day 21.

No clear trends could be derived from the DOC data, however, mean soil DOC concentrations (0-7.5 cm) of all treatments increased (on average), for at least the first 14 days then ranged between 65 and 114 mg C kg dry soil^{-1} from day 14 onwards (Figure 4-2 C). Mean soil DOC concentrations of the 400 mg NH_4^+-N L^{-1} treatment were significantly lower (P = 0.039) than the control and 150 mg NH_4^+-N L^{-1} treatments on day 28 (65 mg C kg dry soil^{-1}), and lower (P = 0.024) than the 90, 150 and 200 mg NH_4^+-N L^{-1} treatments on day 35 (76 mg C kg dry soil^{-1}). The 90 mg NH_4^+-N L^{-1} treatment also had significantly less DOC than the 150 mg NH_4^+-N L^{-1} treatment on day 28 (79 mg kg dry soil^{-1}) and the 300 mg NH_4^+-N L^{-1} treatment had significantly less DOC than the 200 mg NH_4^+-N L^{-1} treatment on day 35 (91 mg kg dry soil^{-1}).

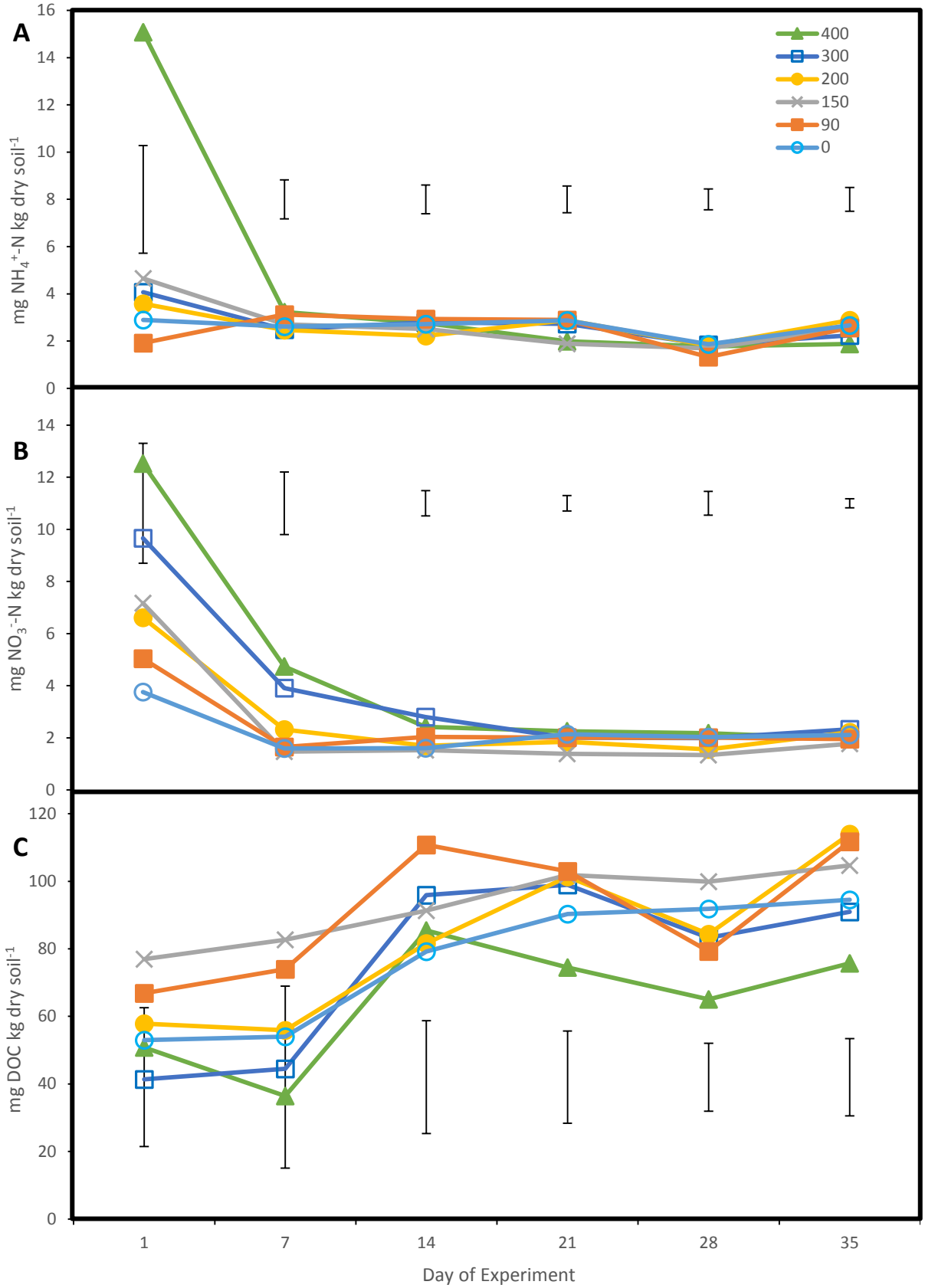

Figure 4-2 Mean (n = 4) soil inorganic-N and dissolved organic-C concentrations 0-7.5 cm depth, over time for the control (0) and FDE treatments (90, 150, 200, 300 and 400 mg NH_4^+-N L^{-1}). Vertical bars are the LSD (P=0.05). A, Ammonium-N (NH_4^+-N); B, Nitrate-N (NO_3^--N); C, Dissolved organic-C (DOC). Note different scales on Y-axis.

4.3 N_2O Fluxes

Emissions from all FDE treatments exceeded those of the control for the first six days following treatment application, returning to near background levels on the 7th day ($P<0.05$) (Figure 4-4). Peak mean N_2O-N fluxes for all treatments were observed on day 1 (c.a. 18 hrs following treatment application), averaging 5.54, 39.0, 48.0, 40.7, 60.9 and 65.2 g N_2O-N ha^{-1} day^{-1} for the control and 90, 150, 200, 300 and 400 mg NH_4^+-N FDE treatments, respectively. FDE treatments did not differ significantly from each other on day 1, however, the 400 mg NH_4^+-N L^{-1} treatment exerted higher fluxes than all other treatments from day's two to five. The 300 mg NH_4^+-N L^{-1} treatment also exerted a significantly higher fluxes than the 90 mg NH_4^+-N L^{-1} treatment on day 5.

Over the 35-day trial period, cumulative N_2O emissions of FDE treatments were significantly greater than the control ($P<0.001$). With the exception of the 200 mg NH_4^+-N L^{-1} treatment, cumulative N_2O-N emissions increased linearly with the increasing rates of NH_4^+-N applied (Figure 4-3). Figure 4-3 shows that 45.9% of the variation in cumulative N_2O emissions is explained by the variation in NH_4^+-N applied ($F_{5,18}=3.05$, $P=0.036$). The 400 mg NH_4^+-N L^{-1} treatment produced 173 g N_2O-N ha^{-1} over the 35 days, significantly more than all other treatments ($P<0.05$).

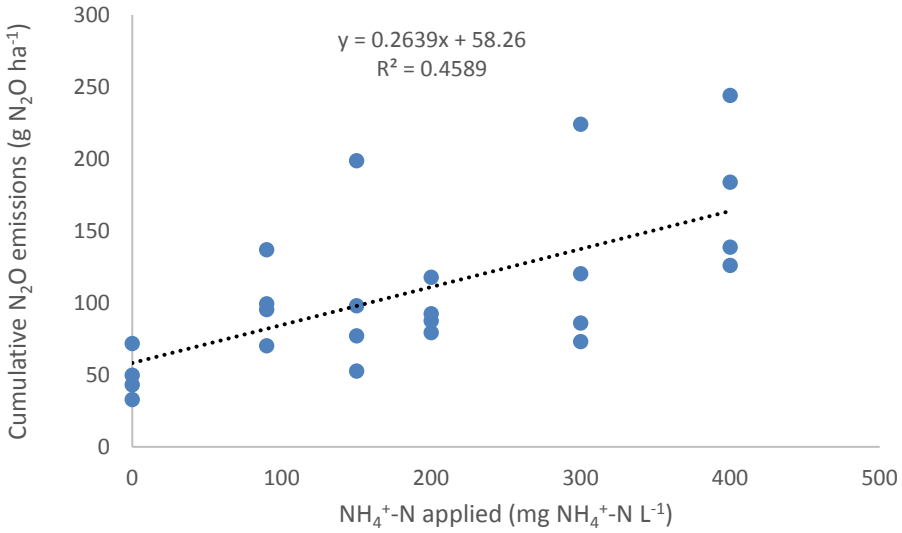

Figure 4-3 Cumulative N_2O-N emissions versus NH_4^+-N content of FDE for the 35-day period following FDE application to perennial ryegrass-white clover pasture (n = 1).

The N_2O EF's of the FDE in terms of % total N applied were not significantly different from each other. However, in terms of % NH_4^+-N, the 90 mg NH_4^+-N L^{-1} treatment had a significantly higher ($P<0.05$) EF (0.56) than the 200, 300 and 400 mg NH_4^+-N L^{-1} treatments (0.22, 0.26 and 0.31, respectively). No clear trends were observed in the EF's of the five treatments (Figure 4-5).

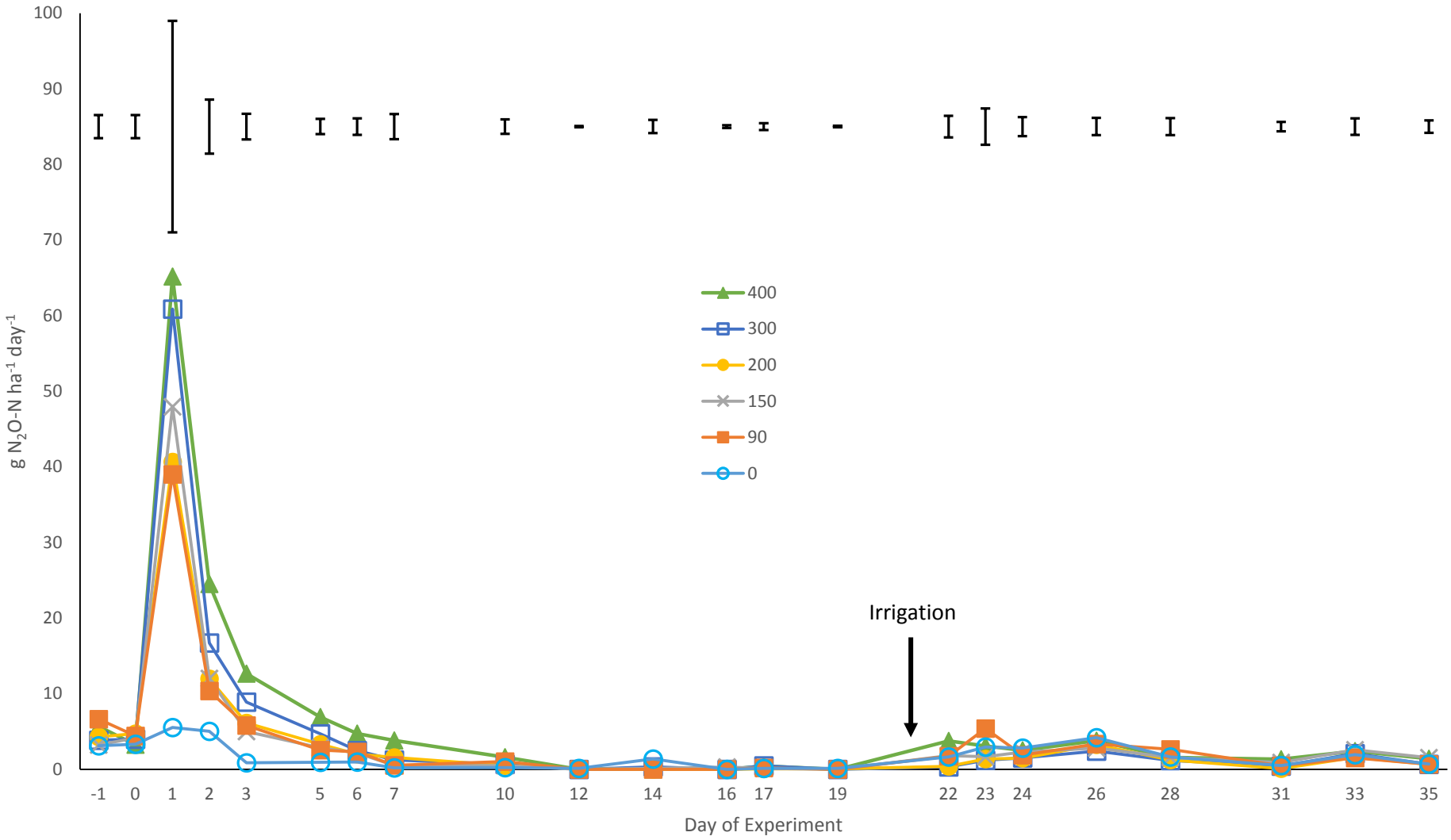

Figure 4-4 Mean (n = 4) N_2O-N fluxes versus time for the control (0) and FDE treatments (90, 150, 200, 300 and 400 mg NH_4^+-N L^{-1}). Vertical bars are the LSD for FDE treatments.

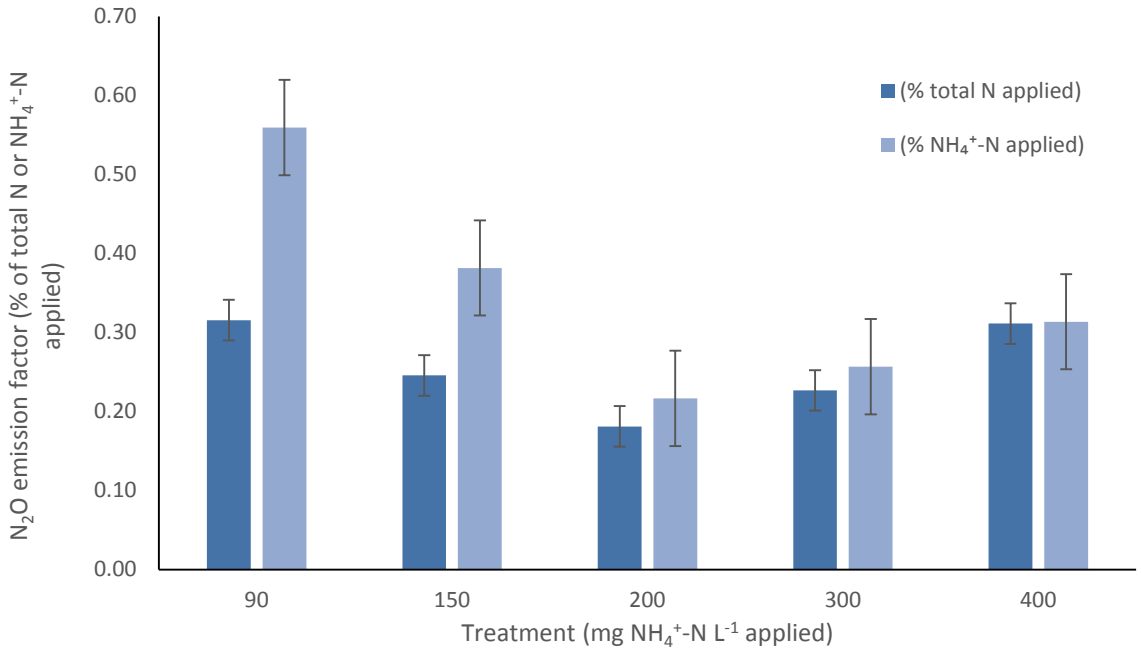

Figure 4-5 Mean (n = 4) N_2O emission factors of FDE in terms of both % N and % NH_4^+-N applied. Error bars are the SEM.

No significant differences in the ^{15}N enrichment of N_2O emissions, relative to the ^{15}N enrichment of the FDE applied, were observed between the 150 and 400 mg NH_4^+-N L^{-1} treatments on days 1 or 2. ^{15}N enrichment of N_2O emissions from the 150 and 400 mg NH_4^+-N L^{-1} treatments were 52 and 54% of the ^{15}N enrichment of the FDE applied, respectively, on day 1 and 33 and 38% of the ^{15}N enrichment of the FDE applied, respectively, on day 2. ^{15}N recovery, as N_2O, from the 150 and 400 mg NH_4^+-N L^{-1} treatments was not significantly different from each other on day 1, however, the 400 mg NH_4^+-N L^{-1} treatment resulted in more ($P<0.05$) ^{15}N being recovered as N_2O than in the 150 mg NH_4^+-N L^{-1} treatment on day 2 (Table 4-2).

Table 4-2 Mean (n = 4) ^{15}N recovery, as N_2O, from the 150 and 400 mg NH_4^+-N L^{-1} treatments on days 1 and 2 of the trial.

	% ^{15}N recovery	
Treatment	Day 1	Day 2
150	0.074	0.008
400	0.052	0.014
	NS	*
LSD (0.05)ɬ	.086	0.005

* $P < 0.05$.

ɬ The least significant difference (LSD) between treatment means is shown for a 5% level of significance, P values > 0.05 = nonsignificant (NS).

4.4 Pasture response to FDE

Cuts 1 and 2 were harvested after 19 and 16 days' growth, respectively. In order to accurately compare the dry matter (DM) production of the two cuts, due to unequal periods of growth, DM production of each cut is reported as kg DM ha^{-1} day^{-1}. Cumulative DM production of the 35-day trial period is reported as kg DM ha^{-1} No significant differences in DM production were observed between treatments for cut 1 and cut 2 (Figure 4-6 A), or when the yields of cuts 1 and 2 were combined (Figure 4-6 B). The 400 mg NH_4^+-N L^{-1} treatment, which contained the highest rate of N applied, produced on average 45.0 kg DM ha^{-1} day^{-1} until cut 1 on the 19th day of the trial, similar to the control, which had no N added and still produced 49.9 kg DM ha^{-1} day^{-1}. However, mean DM production of all plots at cut 2 (71.6 kg DM ha^{-1} day^{-1}) was significantly higher than mean DM production of all plots at cut 1 (51.3 kg DM ha^{-1} day^{-1}) ($P<0.001$).

Similarly, no significant differences in DM% of the pasture were observed between treatments for cut 1 or cut 2 (Figure 4-6 C). The 400 mg NH_4^+-N L^{-1} treatment contained 15.1% DM at cut 1, similar to the control, which contained 16.5% DM. However, mean DM% of cut 2 (16.8%) was significantly higher than cut 1 (15.6%) ($P<0.05$).

No significant differences were observed between the control and FDE treatments (90, 150, 200, 300 and 400 mg NH_4^+-N L^{-1}) for N%, g N per plot, apparent N recovery or apparent NH_4^+-N L^{-1} recovery of the pasture at cut 1 (Table 4-3).

Table 4-3 Mean (n=3) N contents and apparent N and NH_4^+-N L^{-1} recoveries of pasture from the control (0) and FDE treatments (90, 150, 200, 300 and 400 mg NH_4^+-N L^{-1}) at cut 1.

	N content		Apparent N recovery	Apparent NH_4^+-N recovery
Treatment	(%)	(g per plot)	(%)	(%)
0	4.02	0.35		
90	4.67	0.48	75.8	136.3
150	4.47	0.43	32.3	50.0
200	4.41	0.50	55.3	68.4
300	4.36	0.52	45.7	51.3
400	4.67	0.47	28.2	28.0
	NS	NS	NS	NS
LSD (0.05)ɬ	0.846	0.182	92.2	172.3

ɬ The least significant difference (LSD) between treatment means is shown for a 5% level of significance, *P* values > 0.05 = nonsignificant (NS).

No significant differences in the ^{15}N enrichment of pasture, relative to the ^{15}N enrichment of the FDE applied, were observed between the 150 and 400 mg NH_4^+-N L^{-1} treatments at cut 1. ^{15}N enrichment of pasture from the 150 and 400 mg NH_4^+-N L^{-1} treatments were 25 and 28 % of the ^{15}N enrichment of the FDE applied, respectively. At cut 1, ^{15}N recovery of the pasture from the 150 mg NH_4^+-N L^{-1}

treatment was on average (n=3) 22.0% of the ^{15}N applied, not significantly different to the 400 mg NH_4^+-N L^{-1} treatment, which recovered 25.5%.

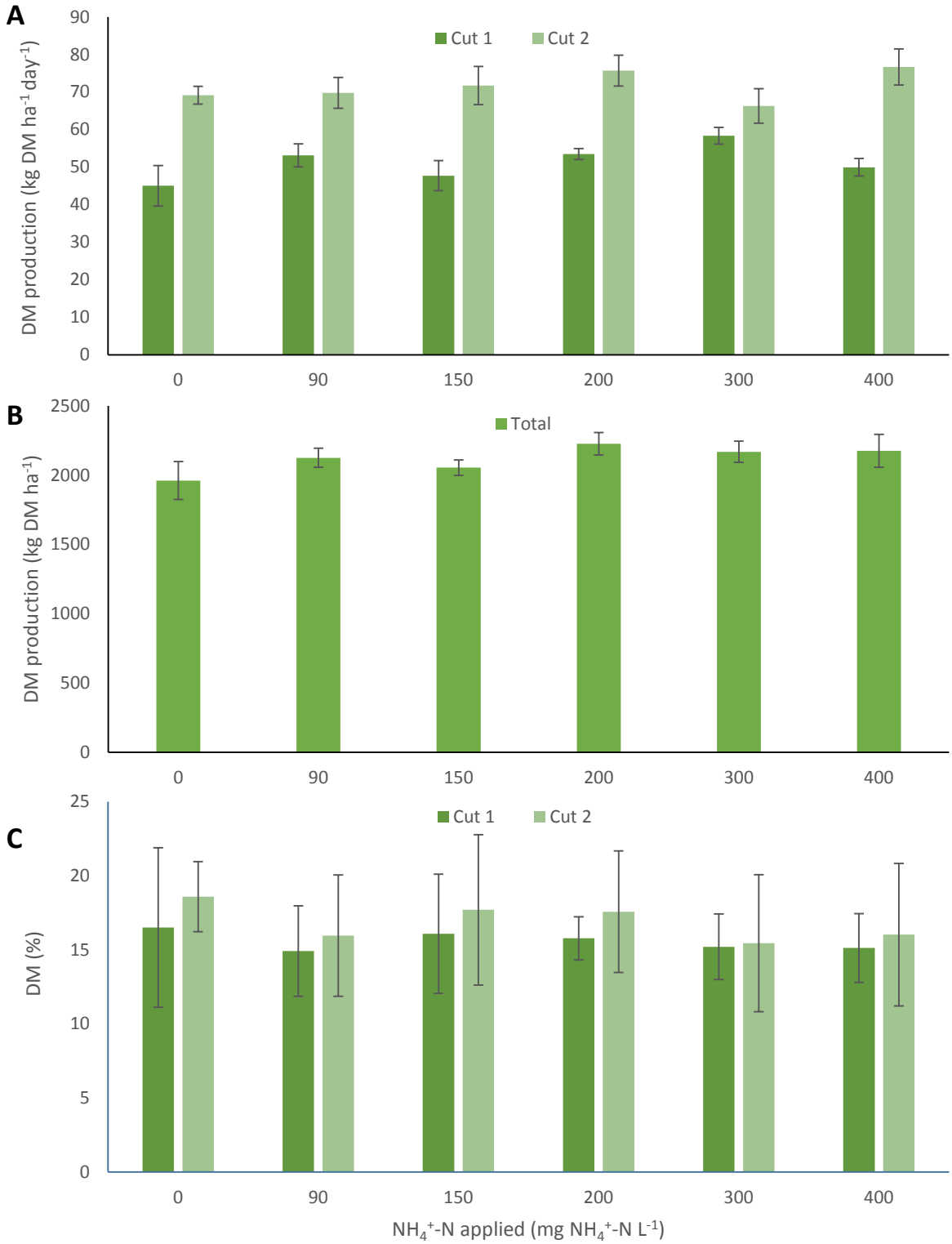

Figure 4-6 Mean (n = 4) Dry matter (DM) production and mean (n = 4) DM% of the control (0) and FDE treatments (90, 150, 200, 300 and 400 mg NH_4^+-N L^{-1}). Error bars are SEM. A; DM production per day (cut 1 and 2), B; Total DM production over the 35-day trial period, C; DM% (cut 1 and 2). Note different scales on Y-axis

Chapter 5

Discussion

In this study farm dairy effluent (FDE) ammonium (NH_4^+-N) concentrations influenced soil nitrous oxide (N_2O) emissions. Cumulative soil N_2O emissions increased linearly with increasing FDE NH_4^+-N concentration (Figure 4-3).

5.1 N_2O Fluxes

5.1.1 Daily and Cumulative Fluxes

Peak emissions of N_2O for all FDE treatments occurred within 24 hrs of their application and were significantly higher than N_2O emissions from the control (Figure 4-4). However, N_2O emissions had declined rapidly by day 2, and returned to background levels by day 7.

Rapid, but short lived, emissions of N_2O from soil following the application of FDE is consistent with the eight New Zealand studies, from which the bulk of our understanding is derived (Barton and Schipper, 2001; Bhandral *et al*., 2010, 2007; Li *et al*., 2016, 2015b, 2014; Luo *et al*., 2008; van der Weerden *et al*., 2016b). The majority of studies observed N_2O emissions peaking within 1 day of FDE application, although peaks were delayed until up to day 6 on some occasions, and N_2O emissions took anywhere up to 1 month, to as little as 24 hrs, to return to background levels. Barton and Schipper (2001), used a more intensive N_2O flux sampling regime, and observed that peak N_2O emissions occurred within 4 hrs of FDE application and returned to background levels within 24 hrs. Thus, it is possible that the peak N_2O flux in the current study occurred prior to or post the first gas sampling 18 hrs after FDE application.

The peak N_2O flux in this study was from the treatment with the highest FDE NH_4^+-N concentration, the 400 mg NH_4^+-N L^{-1} treatment, which produced a mean N_2O flux of 65 g N_2O-N ha^{-1} day^{-1} on day 1 (Figure 4-4). The lowest mean peak flux from FDE treatments of 39 g N_2O-N ha^{-1} day^{-1}, was from the 90 mg NH_4^+-N L^{-1} treatment, which had the lowest FDE NH_4^+-N concentration. Mean cumulative fluxes ranged in magnitude from 101 to 173 g N_2O-N ha^{-1} in the 90 and 400 mg NH_4^+-N L^{-1} treatments, respectively, over the 35-day trial. In comparison, peak and cumulative fluxes from the literature range from 3 to 624 g N_2O-N ha^{-1} -day^{-1} and 10 to 2340 g N_2O-N ha^{-1}, respectively. Therefore, the fluxes observed in this study are at the lower end of the range reported in the literature.

5.1.2 Influence of FDE on soil N_2O emissions

The peak soil N_2O fluxes that were observed shortly after the application of FDE may be due to a number of processes that occur as a result of addition of N and C and an increase in WFPS, from the application of FDE (Barton and Schipper, 2001; Bhandral *et al.*, 2007; Luo *et al.*, 2008b).

Addition of N provides substrate for both dominant soil N_2O sources of nitrification and, following transformations, denitrification (Akiyama *et al.*, 2010; Dobbie and Smith, 2003).

Addition of C not only serves as an electron donor and source of cellular material for denitrification, but it is also an energy source for other microorganisms in the soil, stimulating their activity (Firestone, 1982). The associated respiration and production of CO_2 floods the air-filled pore space, reducing the O_2 concentration, potentially creating anaerobic conditions conducive to denitrification (Firestone, 1982; Rochette *et al.*, 2000).

Meanwhile a number of authors have reported large increases in soil N_2O emissions following an increase in WFPS, particularly when WFPS increased above 60-80% (Ciarlo *et al.*, 2007; Clough *et al.*, 2004; Maag and Vinther, 1996). An increase in WFPS creates anaerobic conditions in the soil, favourable for denitrification (Firestone, 1982).

5.1.3 Sources of N_2O – FDE or soil N, nitrification or denitrification

Nitrate in the FDE was <1 mg L^{-1}, therefore, it is unlikely any significant N_2O emissions were derived from denitrification of this source. Soil inorganic-N concentrations were not measured prior to the application of FDE, however, a soil fertility test determined mineralisable N levels were relatively high (91 mg kg^{-1}) (Table 3-1). Therefore, it is highly likely the addition of C and increase in WFPS from the FDE stimulated denitrification of NO_3^--N already present within the soil, leading to the production of N_2O.

Water-filled pore space of the soil was 68% 14 hrs after treatment application. Clough *et al.* (2004) observed that N_2O emissions, presumably from denitrification, began to increase from a WFPS upwards of 60%. Rabot *et al.* (2015) modelled that the WFPS must be between 62 and 95% for N_2O emissions from denitrification to occur, with the optimum WFPS for peak denitrification between 76 and 79%. Similarly, Ciarlo *et al.* (2007) had observed that peak N_2O emission occurred at a WFPS of 80%. Very high soil moisture levels of approximately >95% retard N_2O diffusion out of the soil and increase the portion of N_2O consumed by reduction to N_2 (Samad *et al.*, 2016). Although not in the peak range for N_2O production, 68% WFPS is still sufficient for N_2O emissions to occur. In addition, the WFPS measurement of 68% was taken 14 hrs after treatment application. Drainage below the 7.5 cm measurement depth may have occurred by this time and therefore, WFPS immediately after

treatment application may have been higher and more conducive to N_2O emissions from denitrification. Furthermore, the WFPS calculated only provides an average for the top 7.5 cm of soil. It is highly likely that moisture was not uniform within the soil, some sites may have had higher WFPS (most likely lower in the soil) while others may have had lower a WFPS.

Balaine *et al.* (2013) found that gas diffusivity (D_P/D_O) was a better predictor of N_2O emissions than WFPS with peak N_2O emissions occurring at a gas diffusivity of 0.006, beyond which N_2O was thought to either become entrapped in the soil, or be further denitrified to N_2. Stepniewski (1981) reported that soils became anaerobic within the range of 0.02 to 0.005, mean modelled gas diffusivities of all plots in this experiment were within this range on day 1 (0.012). This is in support of evidence from WFPS measurements that soil conditions were sufficiently anaerobic for N_2O emissions to occur from denitrification on day 1. In addition, denitrification may have occurred in anaerobic microsites within the soil (Khalil *et al.*, 2004). Therefore, evidence suggests that denitrification of existing soil NO_3^--N contributed to peak N_2O emissions. Both mean WFPS measurements and mean modelled gas diffusivities of all plots of 58% and 0.025, respectively, were in agreement that soil conditions were not conducive to soil N_2O emission from denitrification by day 7.

Both nitrification and denitrification can occur simultaneously, with nitrification occurring in the aerobic zones and denitrification in the anaerobic zones of a soil (Khalil *et al.*, 2004). It is likely that nitrification occurred rapidly following application of FDE. The input of NO_3^--N from the FDE was both negligible (<1 mg L^{-1}), and equal for all of the FDE treatments. However, analysis of soil NO_3^--N concentrations on day 1, 14 hr after treatment application, revealed that treatments applied at a higher NH_4^+-N content also had significantly higher soil NO_3^--N concentrations than those with less NH_4^+-N applied (Figure 4-2). Therefore, it is likely that these differences were due to nitrification of the varying rates of NH_4^+-N (90, 150, 200, 300, 400 mg NH_4^+-N L^{-1}) in the FDE applied. As a result, N_2O production via nitrification or denitrification of the NO_3^--N produced was likely (Kool *et al.*, 2009).

5.1.4 $^{15}N_2O$-N enrichment and source of N_2O

^{15}N enrichment of the FDE applied was 2.79 and 7.25 atom% for the 150 and 400 mg NH_4^+-N L^{-1} treatments, respectively. Mean $^{15}N_2O$-N enrichment of emissions from the 150 and 400 mg NH_4^+-N L^{-1} treatments were 1.49 and 3.80 atom%, respectively, on day 1. This demonstrates that ~54 and ~52% of the N_2O emitted came from the 150 and 400 mg NH_4^+-N L^{-1} treatments, respectively, on day 1. This implies that soil-N and/or mineralization of the unlabelled FDE organic-N contributed to the N_2O flux.

It is unlikely significant immobilization would have occurred as the C:N ratio of both the soil and FDE was 10.6 and in the range of 6.2 to 2.6, respectively, significantly lower than 25, the C:N ratio considered a threshold, above which immobilisation of N occurs (McLaren and Cameron, 1996).

5.1.5 Other reasons for low fluxes

DM production of 49.9 and 71.6 kg DM ha^{-1} day^{-1} for cut 1 and 2, respectively, was in the highest range of DM production quoted by Dairy NZ (2010). Temperature in this study was relatively warm, on average 13°C. This is likely to have encouraged high rates of plant uptake so less N was available for denitrification, an in addition, there would be greater evapotranspiration, reducing the water content of the soil and hence reducing the number of anaerobic sites in the soil for denitrification.

Similarly, the temperature and pH (e.g. Maag and Vinther (1996) and Cai *et al.* (2016); Qu *et al.* (2014) and Samad *et al.* (2016)), have also been shown to influence N_2O emissions.

Relatively dry soils and warm temperatures in the current study were conducive to NH_3 volatilisation, which would have reduced the NH_4^+-N pool for N_2O production. However, New Zealand studies have found only 0.05 to 3.1% of FDE total N is lost to NH_3 volatilisation when applied to soil (Laubach *et al.*, 2015).

As the volume of organic N applied in all FDE treatments was the same, all FDE treatments should have had similar mineralization rates occur, which explains why treatment differences were relatively short lived as any slow release of organic N, later in the trial, would be equal for all treatments. Similarly, lower C:N ratios of treatments with higher rates of NH_4^+-N should not have limited nitrification as chemoautotrophs only need CO_2 (Ferguson *et al.*, 2007).

5.1.6 Emission factors

The FDE EF's in this study, ranging between 0.18 and 0.32% of total N applied, were at the lower range of values reported in the literature of between 0.01 and 4.93% of N applied. The FDE EF's calculated on NH_4^+-N loading in this study were also relatively low. Values calculated from available NH_4^+-N data, where available in the literature, returned EF's of between 0.03 and 4.07% of NH_4^+-N applied, which encompassed the values 0.22 and 0.56% of NH_4^+-N applied calculated in this study.

Again, reduced soil moisture, as measured by WFPS, and relatively low additions of total N and C can be attributed to the low magnitude of the EF observed compared to the literature. In addition, N_2O fluxes were only examined for 35 days in this study, much less than several in the literature. It could be suggested a longer study would capture more emissions, however, as both soil N_2O emissions and soil inorganic-N concentrations had returned to background levels (Figures 4-2 & 4-4), further differences in N_2O fluxes between treatments were not expected.

5.1.7 Irrigation

Application of 20 mm of irrigation on day 21 was insufficient to create anaerobic conditions (modelled gas diffusivity = 0.035), helping to explain the lack of emissions following this attempt to trigger denitrification. Soil inorganic-N concentrations also showed that little NH_4^+-N or NO_3^--N was available for denitrification (Figure 4-2).

5.2 Relationship between FDE NH_4^+-N concentration and N_2O emissions

The initial objective of this study was to determine how FDE NH_4^+-N concentrations influence N_2O emissions.

As hypothesized, N_2O emissions increased linearly with NH_4^+-N application rate, the EF's were unaffected except at the lowest NH_4^+-N application rate, 90 mg NH_4^+-N L^{-1}, which had a significantly higher NH_4^+-N EF, of 0.56% of NH_4^+-N applied. Although not significant, the NH_4^+-N EF's appeared to trend down with NH_4^+-N application rate, up to 200 mg NH_4^+-N L^{-1}, before increasing with further additions of N. This is difficult to interpret, however, it could be due to two separate phenomena. As addition of C and the WFPS were constant for all treatments, this initial decline in the EF could be due to a larger influence of C and WFPS, while the increases at higher rates of N suggest the beginnings of a non-linear relationship.

Treatments in this trial were applied to a constant hydraulic loading to eliminate the effect soil moisture has on N_2O emissions. A 10 mm application depth was typical of the range of values from the literature.

Although total N will still increase at a slow rate due to increases in NH_4^+-N content, no increase in the quantity of organic N will result. Therefore, all increases in N_2O will be due to increases in available N.

5.3 limitations

The linear relationship between NH_4^+-N concentration and soil N_2O emissions may not be true in all situations as the soil was relatively dry during the trial and therefore, soil conditions were largely unfavourable for denitrification and associated N_2O emissions.

Synthetic NH_4^+-N was added to FDE to raise the NH_4^+-N concentration, however, Jost *et al.* (2013) reported that N_2O emissions were positively correlated ($P<0.05$) with FDE microbial biomass. This suggests that it is best to use 100% ruminant FDE, that includes the full endogenous component, where possible, to accurately determine N_2O fluxes from this source.

Chapter 6

Conclusions and Suggestions of Future Research

6.1 Further Research

No matter what regulations and EF's are used to determine application limits and calculate emissions inventories, the values for N (and other components) applied must be accurate to be of use. It has been acknowledged that it is not practical to analyse the N content of effluent before each application, however, it appears that N and NH_4^+-N concentrations of effluent are increasing due to efficiencies in yard wash-down management and as a result, current figures of N inputs used by regulators and scientists may not be valid. Therefore, it is timely and of considerable interest for a wide spread sampling and analysis of effluent to take place. The large range of factors that affect the physical and chemical characteristics of effluent mean an extensive study that incorporated such factors as region and season would be required to be most beneficial. At present, when no clear average values of Total N and NH_4^+-N are available it is difficult to determine what concentrations are most representative and therefore, of most relevance to use for FDE studies.

Investigating the relationship between NH_3 volatilisation and N_2O emissions, especially as the rate of NH_4^+-N applied increases, would be useful to understand the fate of N when FDE is applied to soil.

6.2 Conclusions

- This study provides an insight into the effect of solely increasing the NH_4^+-N concentrations of FDE on N_2O emissions.
- Ammonium concentration is a key driver of N_2O emissions.
- As hypothesized, N_2O emissions observed in this study indicated a positive linear relationship with NH_4^+-N concentration, when FDE was applied at N loading rates below the recommended maximum.
- Further studies that analyse the influence of NH_4^+-N concentrations on soil N_2O emissions in a range of typical environments are required to fully understand this relationship.
- It is likely the response of soil N_2O emissions to increases in FDE NH_4^+-N concentration is influenced by environmental conditions, therefore, the use of a single relationship between the two variables for all environmental situations may not be possible.
- In this trial the EF from FDE ranged from 0.18 to 0.32% of total N applied, significantly less than both those used to calculate NZ's greenhouse gas inventory and those of urine patches and N fertiliser.
- It could also tentatively be concluded that; the recommended best practice maximum FDE N application rate of 50kg N ha^{-1} is not only a suitable limit for NO_3^--N leaching, but also a suitable limit for N_2O emissions, as the portion of N loss from FDE was unaffected by the typical range of NH_4^+-N concentrations up to this limit.
- Intensification of dairy farming in New Zealand, that appears to increase the NH_4^+-N concentration of FDE, will lead to increased N_2O emissions when the FDE produced is applied to soil.

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